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Dry-contact microelectrode membranes for wireless detection of electrical phenotypes in neonatal mouse hearts

Yu Zhao · Hung Cao · Tyler Beebe · Hemin Zhang · Xiaoxiao Zhang · Honglong Chang · Oscar Scremin · Ching-Ling Lien · Yu-Chong Tai · Tzung K. Hsiai

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Abstract Continuous monitoring of aberrant electrical rhythms during heart injury and repair requires prolonged data acquisition. We hereby developed a wearable microelectrode membrane that could be adherent to the chest of neonatal mice for *in situ* wireless recording of electrocardiogram (ECG) signals. The novel dry-contact membrane with a meshed parylene-C pad adjacent to the microelectrodes and the expandable meandrous strips allowed for varying size of the neonates. The performance was evaluated at the system level; specifically, the ECG signals (μV) acquired from the microelectrodes underwent two-stage amplification, band-pass filtering, and optical data transmission by an infrared Light Emitting Diode (LED) to the data-receiving unit. The circuitry was prototyped on a printed circuit board (PCB), consuming less than 300 μW, and was completely powered by an inductive coupling link. Distinct P waves, QRS complexes, and T waves of ECG signals were demonstrated from the non-pharmacologically sedated neonates at ~600 beats per minutes. Thus, we demonstrate the feasibility of both real-time and wireless monitoring cardiac rhythms in a neonatal mouse (17–20 mm and <1 g) via dry-contact microelectrode membrane; thus, providing a basis for diagnosing aberrant electrical conduction in animal models of cardiac injury and repair.

Keywords Neonatal mice · Wireless monitoring · Dry-contact electrodes · ECG · Heart regeneration

Yu Zhao, Hung Cao and Tyler Beebe contributed equally to this work.

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Y. Zhao · X. Zhang · Y.-C. Tai
California Institute of Technology, Pasadena, CA, USA

H. Cao · T. K. Hsiai (✉)
Division of Cardiology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90095, USA
e-mail: THsiai@mednet.ucla.edu

T. Beebe · T. K. Hsiai
Department of Bioengineering, UCLA School of Engineering & Applied Sciences, Los Angeles, CA 90095, USA

H. Zhang · H. Chang
Northwestern Polytechnical University, Xi'an, China

C.-L. Lien
Children Hospital Los Angeles, Los Angeles, CA, USA

O. Scremin · T. K. Hsiai
Research Services, Veteran Affairs Greater Los Angeles Healthcare System, Los Angeles, CA, USA

1 Introduction

Wireless technology has transformed medical diagnosis, monitoring, and intervention to patient-centered care (Bui and Fonarow 2012; Cao et al. 2013). Developing low-cost sensors and wireless systems facilitates constant and pervasive monitoring in a point-of-care environment. However, developing small, wearable, and wireless devices for investigation of tissue repair and regeneration remains an unmet clinical challenge.

Myocardial infarction results in an irreversible loss of cardiomyocytes. Human hearts heal injuries by scarring, which leads to remodeling and heart failure. Unlike adult mammalian hearts that have a limited capacity to repair after injury (Bergmann et al. 2009; Bersell et al. 2009; Hsieh et al.

2007), those of certain fish and amphibians maintain a regenerative capacity throughout adult life. This capacity has spawned numerous animal studies to investigate the underlying mechanisms for tissue repair in humans (Huang et al. 2012; Kikuchi et al. 2010; Lien et al. 2012; Narula et al. 1996; Olivetti et al. 1997). Through genetic fate mapping, Porrello et al. recently discovered the transient regenerating capacity of 1-day-old neonatal mice in response to heart injury, and this capacity is lost by 7 days of age (Porrello et al. 2011). Whether aberrant cardiac electrical rhythms developed during heart regeneration remained unknown.

To monitor the cardiac electrical activities, we have previously established the use of microelectrodes to elicit aberrant electrocardiogram (ECG) signals. We assessed arrhythmia in the zebrafish model of heart regeneration, and demonstrated that ventricular repolarization (QTc intervals and T waves) failed to normalize despite histological evidence of a fully regenerated myocardium (Cao et al. 2014; Yu et al. 2012). This observation suggested that delayed expression of ionic channels for cardiac conduction may be implicated in the prolonged QTc intervals (Yu et al. 2010). Thus, a real-time approach to monitor ECG signals from the mammalian model of heart injury and repair holds promise to detect cardiac arrhythmias.

To establish this strategy, we addressed three bioengineering challenges: 1) the dry-contact recording electrodes were adhered to the neonatal chest free of gel or adhesive via surface tension; 2) mechanical anchors were designed adjacent to the dry-contact electrodes for firm contact between the electrodes and the non-planar body surface for ECG recording from the hearts; and 3) an expandable fixation accommodated in response to varying animal size. Here, we demonstrate the feasibility of a wearable and wireless strategy to achieve real-time monitoring of cardiac conduction; thereby, paving the way for detecting potential aberrant electrical signals in the setting of heart injury and repair.

2 Designs and implementations

Despite decades of developments in dry-contact electrodes (Chi et al. 2010), their sensitivity has been influenced by noise and motion for biomedical applications. Here, novel strategies to enhance the signal-to-noise ratios (SNR) were developed at three different levels: sensor, system and algorithm. At the sensor level, the aforementioned mechanical anchors and fixations were incorporated for positioning the electrodes to the anatomic regions. In response to the neonatal growth, the expandability of the fixation was designed to adapt to the increase in size. At the system level, the post-processing circuitry was implemented with high input-impedance and low input-noise to compensate for the high interface impedance and noise level in association with the dry-contact electrodes. At the algorithm level, wavelet analysis and noise-reduction technique further increased the SNR at the receiver side.

2.1 The microelectrode membrane and mechanical fixture

In preparation for the long-term recording requirement, parylene C was chosen as the substrate and insulating material by virtue of excellent biocompatibility (*FDA APPROVED, USP class VI*) and low water, gases and ions permeability. The microelectrode membrane was manufactured using micromachining techniques. Titanium/Gold electrodes were sandwiched between two layers of parylene C, and were exposed to oxygen plasma etching at the recording sites and connecting pads. Four gold electrodes with a diameter of 200 μm were linearly arranged (Fig. 1). The reference electrode was placed 5 mm apart from the recording electrodes. There were four connecting pads assembled to a customized zero-insertion-force (ZIF) cable end by conductive epoxy and encapsulated by biocompatible silicone. The membrane was measured ~10 μm in thickness and the weight was less than 0.5 mg. After an annealing process in the vacuum oven at

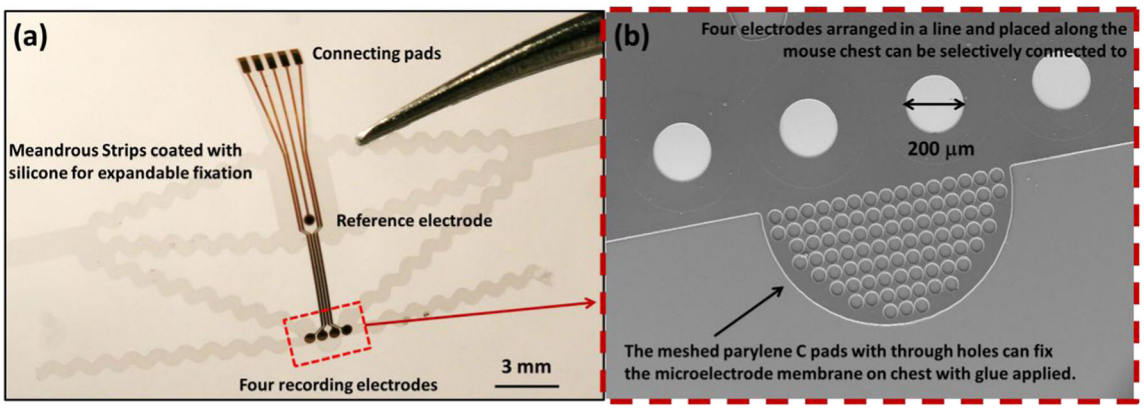

Fig. 1 **a** The microelectrode membrane was embedded with recording electrodes, reference electrode, connecting pads to the post-processing circuitry, meshed parylene-C pad for anchoring and meandrous strips for expandable fixation. **b** The Scanning Electron Microscope (SEM) photo revealed four titanium/gold microelectrodes and the meshed parylene-C pad with through holes

200 °C for 2 days, the membrane was curved to conform to the surface curvature of the neonate chests.

For safety and comfort to the neonates, the dry-contact electrodes operated in the absence of electrolytes. The impedance at skin-electrode interface was modeled as a resistor in series with a combination of parallel resistor-capacitor. At the frequency ranging from 2 to 125 Hz (the frequency range of interest for ECG characteristics), the model was simplified as an interface capacitor.

Low-power oxygen plasma was applied to roughen the microelectrode surface, allowing for an increase in the effective contact area and a decrease in the interface impedance while still maintaining a high spatial resolution. The introduction of a first-stage amplifier with high input impedance rendered the microelectrode interface impedance negligible. As a result of the thin layer of micro-fabricated metal cables (0.2 μm in thickness) running in parallel and being connected to the input-stage amplifier, the shunt capacitance was also negligible.

The central portion of the microelectrode membrane was designed with stretchable parylene-C strips for securing to the abdomen. The strips and sharp edges of the entire membrane were coated with the biocompatible silicone to match with the Young's modulus of the animal skin. A mechanical mismatch would result in cell and tissue damage and possibly infection. In addition, a meshed parylene-C pad adjacent to the microelectrodes served as an anchor (Fig. 1b). The applied surgical glue permeated throughout the distributed holes, providing contact between the parylene-C embedded microelectrodes and the animal chest. This unique design minimized electrical signal fluctuations from respiration-induced mechanical noise, and reduced the interface impedance of dry-contact electrodes.

2.2 The signal processing circuitry and wireless power link

The signal amplification and filtering were critical to address the small surface ECG signal strength (<150 μV) and frequency range (2–125 Hz). The signal processing circuitry required the two-stage amplifiers and a band-pass filter prior to transmitting the signals to the data receiver. The ECG signals were passed through a first-stage instrumental amplifier (*INA333 from Texas Instruments*) to provide high input-impedance, and then to an operational amplifier (*OPA333 from Texas Instrument*) to allow for a band–pass filter and secondary amplification. High-pass and low-pass filters filtered out signals with frequency below 2 Hz and above 125 Hz. The circuit communicated with the external data acquisition and process units via an infrared Light Emitting Diode (LED). There was an approximately 2 cm distance between the transmitter and receiver. The LED transmitted an analog signal which was digitized on the receiving end. The circuit design highlighted the high-pass and low-pass filters in relation to the two-stage amplifiers (Fig. 2). The implemented circuitry featured off-the-shelf ICs and individual components on a commercial PCB. The microelectrode membrane was inserted into the zero-insertion-force (ZIF) connector on board. Single-channel wireless ECG signal recording was established between one of the four front electrodes and the reference electrode.

During operation of the system and the load, a DC voltage of 1.8 V or above and an average power of 300 μW were required. In that case, the equivalent load was calculated to be 20 kΩ. A 2-coil inductive link coupled in a solenoid configuration was designed to provide power continuously. A miniaturized and high-quality-factor receiver power coil was constructed by wrapping 30/48 Litz wires around a Ni/Zn ferrite core to establish high magnetic permeability and low electrical loss. The secondary coil had 8 turns, resulting in an outer diameter of 1.2 mm, an inner diameter of 0.7 mm, and a length of 4 mm. For the target frequency range, an inductance of 143 nH and a quality factor of 22.6 around 10 MHz were achieved to enhance the power transfer efficiency of 1.4 % from 9 to 15 MHz. In this context, an optimal operating frequency of 11.1 MHz was chosen.

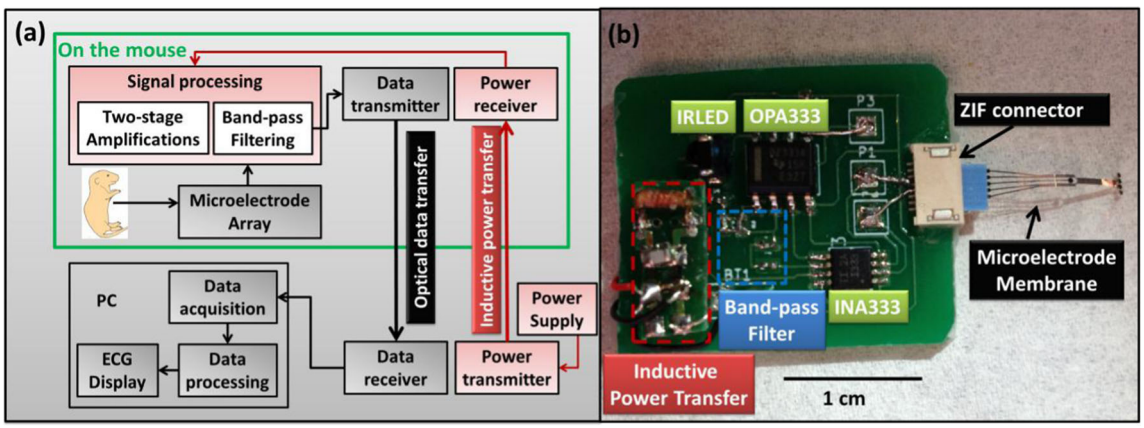

Fig. 2 **a** The schematic diagram of the wireless ECG recording system includes the wearable part carried by the live animals and the external part for data acquisition and power transfer. **b** The microelectrode membrane was integrated with the printed circuit board

2.3 The wavelet transform

In addition to the pre-amplification and filtering on board, wavelet transform was performed to enhance the signal-to-noise ratio (SNR). The signals were divided into six frequency segments ranging from 0 to 125 Hz. A pre-selected threshold value was applied to each individual frequency segment to suppress noise while retaining the signal components as previously described (Cao et al. 2014; Yu et al. 2010).

3 Experiments

The wireless studies on the neonatal mice were performed in accordance with the Institutional Animal Care and Use Committees (IACUC) at Children Hospital Los Angeles, Los Angeles, CA, USA. The animal experiments were performed in compliance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

The microelectrode membrane was evaluated at the system level. The signal processing and wireless communication circuitry prototyped on the printed circuit board (PCB) includes two-stage amplification, band-pass filtering, inductive power transfer, and optical data transmission (Fig. 2). Despite a low-power-consumption circuitry, wireless power transfer instead of battery has remained to support the prolonged recording.

For wireless ECG recording, 12 different neonatal mice aged between 1 and 7 days old were positioned on a baseplate without sedation. The PCB was first gently fixed on the abdomen by thin strips of medical tapes, followed by adhering the microelectrode membrane to the chest. Surgical glue (*Dermabond Advanced*™) was applied to the anchoring meshed pad and permeated through the holes. The mouse lying on baseplate was then placed inside the power- transmitting coil with a diameter of 2.8 cm. When the power was activated to the transmitting coil, real-time ECG signals were acquired (Fig. 3).

4 Results and discussions

Reproducible trials on neonatal mice were performed to reveal distinct ECG signals in the presence and absence of pharmacological sedation. In Fig. 4a, a comparison in ECG signals between the non-sedated and sedated conditions highlighted the distinct heart rates and ECG repolarization patterns. A reduction in the heart rates by 50 % was noted in the presence of sedation (80 μg/gr body weight Ketamine/xylazine). With the mechanical interference during recording and the signal attenuation during transmission, the P waves, QRS complexes and T waves remained distinct despite a reduction in SNR without sedation (Fig. 4b).

The unique aspect of our wireless approach lies in the signal processing circuitry and optical wireless data communication. For the first time, we have provided electrophysiological signals from a neonatal mouse model of heart regeneration in the absence of sedation (Fig. 3b). The capability to monitor intrinsic heart rates and electrical signals without exogenous neurologic influence opens a new avenue for cardiac and neurologic dug screening, disease modeling, and toxicity studies.

The recent advance in stem cell-based therapy warrants a non-invasive and long-term strategy to assess electrical and mechanical coupling to the host hearts. Towards this end, our wearable and wireless ECG monitoring system would further offer a non-invasive approach to address maturation and integration of the stem cell-derived cardiomyocytes. The conformity, light weight, and high spatial resolution of the microelectrode membrane would further bridge wireless technology with pre-clinical animal models of heart regeneration with a translational implication in addressing human cardiac repair.

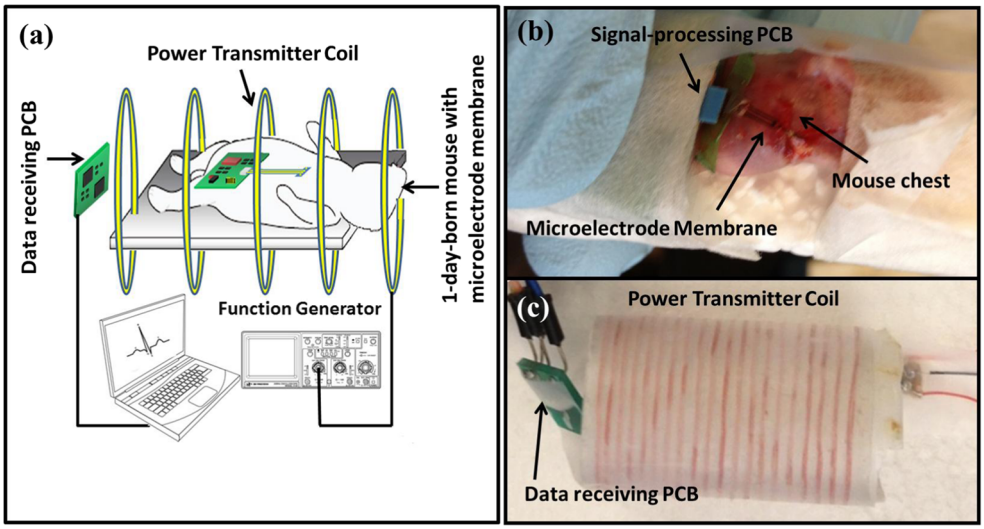

Fig. 3 **a** The experiment setup of the wireless ECG recording illustrates the neonatal mouse positioned on the baseplate enclosed by the transmitting coil. **b** During the surgery, the microdevice was anchored on the neonate. **c** The transmitter coil surrounded the neonate

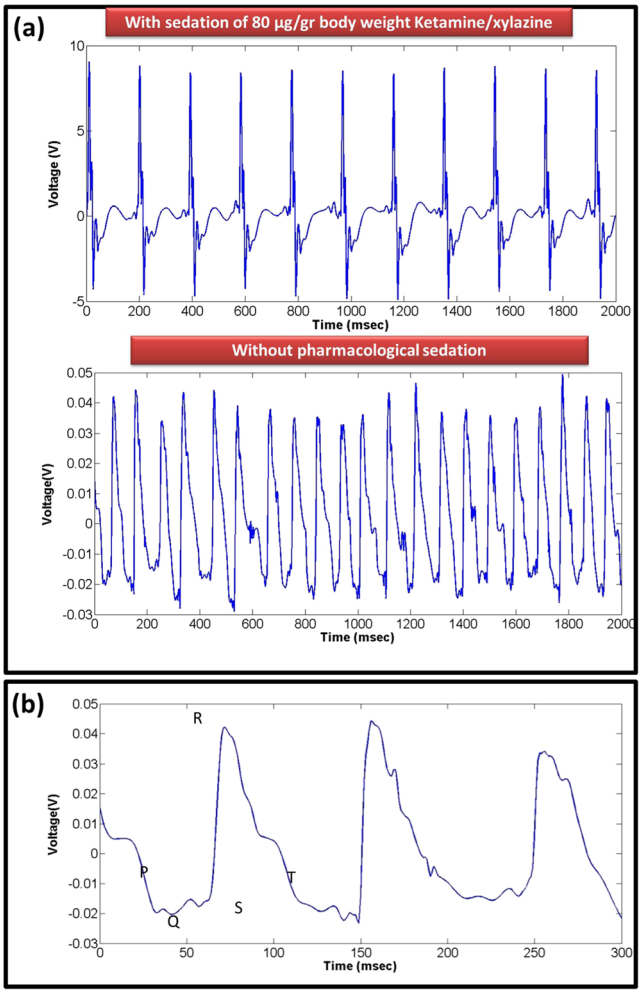

Fig. 4 **a** A comparison of cardiac rhythms revealed a heart rate of ~300 bpm under pharmacological sedation and ~600 in the absence of sedation. **b** A representative ECG signals highlighted the distinct P waves, QRS complexes and T waves

While histological staining was typically used to characterize the heart injury and regeneration process, real-time and long-terms ECG monitoring using the microelectrodes could reveal aberrant electrical conduction, or arrhythmia, in the injured and regenerating myocardium otherwise difficult with the conventional technology (Yu et al. 2012). For instance, using the zebrafish model of heart regeneration, we reported that ventricular repolarization (ST intervals and T waves) failed to normalize despite histological evidence of fully regenerated myocardium at 60 days post ventricular amputation. Our finding suggested further cardiac remodeling may be required to fully integrate regenerating myocardium with host myocardium (Yu et al. 2010). However, the previous ECG recording required pharmacological sedation (*Tricaine*) thus affecting the cardiac pacemaker cells (Sun et al. 2009; Yu et al. 2010); resulting in a reduction in the mean heart rate and atrial arrhythmia (Sun et al. 2009). In this context, a wearable and wireless strategy would advance the field of regenerative medicine in the small animals and for wound repair in humans.

The small size and constant movements of the small animals pose a challenge to implement a wireless ECG system. Here, we developed conformable sensors with biocompatibility and flexibility. We demonstrated a dry-contact membrane to secure the sensors for stable and real-time ECG monitoring. Furthermore, we designed a recording circuitry operating on wireless power. The integrated and packaged system enabled the realization of a compact and lightweight membrane for both small animal models and future individualized patient care.

To our best knowledge, we have addressed the bioengineering challenges for wireless monitoring of electrical phenotypes from a neonatal mouse (17–20 mm and <1 g) for the laboratory environments (Kulandavelu et al. 2006). Our data demonstrated the feasibility of seamless adherence to the ventral surface of the small animals for wireless acquisition of ECG; thus, providing a basis for on-line and off-line strategy for mobile-health. However, our current data focused on the design and testing of the paraylene-C based strategy. Demonstration of 7-day duration would require the assessment of the shift in electrode position in response to increasing animal size. In addition, long-term monitoring would require us to address animal behavior in response to device-mediated irritation and scratching, and to prevent the mother's attempt to remove the membranes from her litter mates. In terms of power transfer, the neonate was contained in a small cage surrounded by the coil for inductive coupling. Translating to clinical applications would require long-term and statistical data analysis. Nevertheless, we envision that inductive power transfer would be employed for the patients lying on the hospital bed where the inductive coils could be embedded in the gurney rails in close proximity to the electrode membrane adhered to the patients.

5 Conclusion

For the first time, we have provided electrophysiological signals from a neonatal mouse model in the absence of pharmacological interference to measure the intrinsic heart rates. We have demonstrated a wearable and wireless microelectrode membrane design to enable real-time monitoring of cardiac rhythms. The membrane's conformity to the non-planar recording surface and the expandability to the varying size of the animals provide numerous translational implications for *in-situ* detection of aberrant electrical activities for cardiac and neurological drug screening, disease and trauma modeling, and toxicity studies.

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