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Wearable Multi-Channel Microelectrode Membranes for Elucidating Electrophysiological Phenotypes of Injured Myocardium

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Abstract

Understanding the regenerative capacity of small vertebrate models has provided new insights into the plasticity of injured myocardium. Here, we demonstrated the application of flexible microelectrode arrays (MEAs) in elucidating electrophysiological phenotypes of zebrafish and neonatal mouse models of heart regeneration. The 4-electrode MEA membranes were designed to detect electrical signals in the aquatic environment. They were micro-fabricated to adhere to the non-planar body surface of zebrafish and neonatal mice. The acquired signals were processed to display electrocardiogram (ECG) with high signal-to-noise-ratios, and were validated via the use of conventional micro-needle electrodes. The 4-channel MEA provided signal stability and spatial resolution, revealing the site-specific electrical injury currents such as ST-depression in response to ventricular cryo-injury. Thus, our polymer-based and wearable MEA membranes provided electrophysiological insights in long-term conduction phenotypes for small vertebral models of heart injury and regeneration with a translational implication for monitoring cardiac patients.

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Author Contribution

H. Cao, Y. Zhao and T. K. Hsiai wrote the manuscript. Y. Zhao fabricated the device. H. Cao, F. Yu, Y. Zhao, J. Lee and A. Darehzereshki performed the experiments. H. Cao processed the data. T. K. Hsiai, Y.-C. Tai, N. C. Chi and C.-L. Lien provided advice and discussion. T. K. Hsiai and Fei Yu conceived the design, developed the IACUC protocol, and implementation of the study. Y. C. Tai advised and supervised the microfabrication and validation of the equivalent circuit. All authors reviewed the manuscript.

Competing Financial Interests

No competing financial interests exist.

Keywords

Multi-channel flexible microelectrode array (MEA); heart injury and regeneration; zebrafish; neonatal mice

1. Introduction

In the era of regenerative medicine, flexible membranes containing microelectrode arrays (MEAs) hold promises for real-time and long-term monitoring of aberrant electrical currents in injured and regenerating organs and tissues. Epidermal electronics elucidated electroencephalography (EEG) signals from the brain of the rat model [1]. Implantable MEAs were able to reveal the feasibility of assessing functional deficits underlying traumatic spinal injuries [2], and to stimulate photoreceptors underlying visual impairment [3]. Flexible MEAs were also reported to synchronize with myocardial Calcium transients in the zebrafish hearts [4]. However, whether wearable MEA membranes are able to uncover site-specific electrical phenotypes for an extended period during myocardial repair in response to injury has not been demonstrated.

Myocardial infarction results in irreversible loss of heart tissues or cardiomyocytes [5]. Injured human hearts heal by scarring, which leads to remodeling and subsequently, heart failure [6]. Unlike adult mammalian tissues, certain fish and amphibians maintain a regenerative capacity throughout adult life. The conventional view of mammalian hearts as having virtually no regenerative capacity is now questioned by recent animal and human studies, in which new cardiomyocytes may arise from existing cardiomyocytes and progenitor or stem cells [7–11]. Zebrafish (*Danio rerio*) hearts fully regenerate after 20% of ventricular resection [12], thereby providing a genetically tractable system for drug discoveries and inherited cardiac arrhythmias [13]. Through a genetic fate mapping, Porrello et al. further reported heart regeneration in 1-day-old neonatal mice after injury, but this regenerative capacity was lost at day 7 after birth [14]. Thus, the advent of MEA to constantly monitor electrical currents of the injured hearts with a limited regenerative capacity is engendering enthusiasm [15].

Atrial and ventricular ECG signals in adult zebrafish were akin to those of humans [16]. Distinguishable P wave and QRS complex were demonstrated by using oral perfusion to maintain oxygenation and muscle paralytics to reduce mechanical noise [17]. Developmental cardiac electrical signals from Zebrafish larvae to adults were uncovered between 7 and 14 days post fertilization (dpf) via micro-electrode pipette [18, 19], and adult ECG signals were acquired via a pair of micro-needle electrodes [20]. To capture aberrant electric conduction signals without removing the fish from water or disturbing the neonatal mice from feeding, we designed wearable MEA membranes for long-term monitoring. The multi-channel MEA membranes were micro-fabricated with gold electrodes embedded in a wearable parylene-C jacket, and were designed to adhere to the chest of animals. The aberrant electrical phenotypes; namely, ST-depression, were detected in response to cryo-injury of the zebrafish myocardium. Despite the swift movement of the fish, the MEA membrane remained adhered and functional in the aquatic environment. Hence, we demonstrate a novel application of water-resistant and wearable multi-channel MEA

membranes to elucidate electrophysiological changes in response to tissue injury in zebrafish and mice with a translation implication for monitoring cardiac patients.

2. Design and Methods

a. Microelectrode array (MEA) fabrication

MEAs were micro-fabricated to enable biocompatible and flexible membranes that could be conformed to non-planar anatomical subjects. A 5 μm -thick parylene-C layer was first deposited onto a hexamethyldisilazane-treated (HMDS) silicon wafer. Next, a double layer of Au (0.2 μm) on Ti (0.02 μm) was deposited by thermal evaporation and patterned by a regular gold etchant. After another 5- μm thick parylene C was deposited, the recording sites and connecting pads were exposed by oxygen plasma etching. Four working electrodes were positioned to the caudal region of the MEA while the reference electrode was in the mid-section (Fig. S1). The connecting pads were electrically connected to a customized flexible flat cable (FFC) via conductive epoxy, and polydimethylsiloxane (PDMS) was applied to establish electrical insulation and mechanical strength.

In the case of zebrafish, additional wing components were implemented to facilitate long-term adherence (Figs. 1a, S2a). The electrodes in the caudal region of MEA were thermally annealed to allow for adhering to the epicardial region of zebrafish. A pair of wings were circumferentially enclosed around the fish, and were dorsally connected via epoxy (Vetbond, 3M Animal Care Products, St. Paul, MN, USA) (Fig. 1b). The relative dimension of the reference electrode to the working electrodes (50, 100, 200 and 300 μm in diameter) were easily modified to optimize contact area with the non-planar anatomy.

b. Characterization of the implantable membrane electrodes

A pair of gold (Au) planar electrodes formed a transducer for measuring the ionic potential difference between working and reference points (Fig. S2). In light of the specific dimensions and the frequency range of ECG (2 to 125 Hz), the metal-tissue interface was modeled as a double-layer capacitor in parallel with a resistor (Fig. S2b). The impedances of electrodes with different diameters were characterized in the 0.7% saline solution with sufficient conductivity (Impedance Analyzer, Gamry Instruments, Warminster, PA, USA). The impedance of the electrode with a diameter of 300 μm was less than 1 $\text{M}\Omega$ at 100 Hz (Fig. S2d).

The equivalent circuits were composed of the front-end planar metal electrodes in direct contact with signal source and the back-end instrumentation amplifier with high input impedance. The electrical conduction of the heart muscle cells was modeled as a battery with ion exchange. The electrical property of the surrounding tissues was modeled as a network of resistors. Electrodes converted the ionic signals generated by the cardiomyocytes into electrical signals which were amplified and processed. The working electrodes were placed closer to the signal source than the reference electrode, and all electrodes were equivalently inserted into different points of the resistor network. One of the working electrodes (A, B, C or D) and the reference electrode were positioned proximally to the hearts and towards the tail, respectively (Fig. S2a). The shunt capacitors between the recording cables remained small and negligible as compared to the electrode impedances

and amplifier input impedance. The input impedance of instrumentation amplifier was as high as a 1000-Gohm resistor in parallel with a 5-pF capacitor. The impedance of the working electrode with a diameter of 300 μm versus the reference electrode remained below 1 M Ω at 100 Hz, enabling a significant voltage drop across the instrumentation amplifier. To minimize decoupling through the low-impedance path formed by the fish body, we positioned the electrodes proximal to the source of cardiac conduction. Table S1 highlights the components in the equivalent circuit.

c. Real-time acquisition of electrical conduction in zebrafish and neonatal mice

All the experiments on zebrafish and neonatal mice were performed in accordance with the Institutional Animal Care and Use Committees (IACUC) at University of Southern California (USC) (IACUC with permit number #11110). All experiments were performed with efforts to observe animal discomforts, and animals were euthanized for signs of suffering in compliance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Electrical phenotypes in injured zebrafish—Sedated adult zebrafish (in 0.04% Tricaine methane sulfonate - Tricaine) were placed on a damp sponge for microscopic procedures as previously described [18, 20]. A 2-mm-long horizontal incision was created at 0.5 mm caudal to the heart. The MEA head containing four working electrodes (WE) was inserted into the epicardium while the reference electrode (RE) was anchored towards the fish tail. The pair of wings was dorsally adhered as described above. The fish were allowed to recover in water free of Tricaine (Figs. 2a and 2b).

During electrical signal acquisition, the sedated fish implanted with the MEA was placed in a Faraday cage (Fig. 2c). The signals were amplified by 10,000-fold (A-M Systems Inc. 1700 Differential Amplifier, Carlsborg, WA), and filtered between 0.1 and 500 Hz and at a cut-off frequency of 60 Hz (notch). The filtered signals were digitized at a sampling rate of 1,000 Hz (National Instruments USB-6251 DAQ device, Austin TX, and LabVIEW 8.2), and the signal-to-noise ratio (SNR) was enhanced by using the both wavelet analysis and noise-reduction techniques as previously described [4].

ECG acquisition in neonatal mice—The mice were anesthetized by a subcutaneous injection of 80 $\mu\text{g}/\text{gr}$ body weight Ketamine/xylazine, and the caudal portion of MEA membrane was adhered to the chest (Fig. 2d). To enhance SNR, we employed a needle electrode for reference to reduce the interfacing impedance. The signal processing was performed as described for the zebrafish.

Heart injury procedures—Wild type adult zebrafish (subjects were bred and raised in our laboratory from parents purchased at Tong's Tropical Fish and Supplies, Los Angeles, CA, USA) and one-day old neonatal mice (ICR/CD-1 strain, Charles River Laboratories, MA) underwent ventricular cryo-injury via liquid nitrogen-chilled metal probes. The sedated zebrafish were mounted on a damp sponge under a stereo microscope for an open-chest procedure. Ventricular injury was induced by applying a liquid-nitrogen-chilled metal probe (diameter 0.8 mm) for 24 seconds. The fish was returned to the fresh water for recovery.

After 3–7 days, the fish underwent MEA implantation. Neonatal mice were cryo-injured by employing the similar procedure. After 7 days, the mice underwent ECG signal acquisition.

3. Results

a. Cryo-injury induced aberrant electrical phenotypes in both neonatal mice and zebrafish

Using the wearable MEA membrane, we demonstrated ECG data acquisition prior to and after signal-processing in the neonatal mouse hearts on day 7 after cryo-injury (Figs. 3b and 3c). Histology of the cryo-injured myocardium was compared with the sham neonatal mouse hearts (Fig. 3a). Signal processing via wavelet transform enhanced the signal-to-noise ratio (SNR) (Fig. 3c with MEA and 3d with a needle electrode). The MEA-acquired electrical signals were further validated with the micro-needle electrodes (shown as processed data in Fig. 3d). The SNRs were calculated to be 12.6 dB for the raw (3b) and 20.8 dB for the processed data (3c), respectively. While the needle electrodes revealed the similar ECG patterns, the 4-channel MEA membrane further uncovered the T waves and ST-depression corresponding to the site-specific injury currents, otherwise under-detected by the individual micro-needles (Figs. 3).

b. Electrical signal acquisition in aquatic environment via MEA membranes

To demonstrate the feasibility for long-term ECG monitoring in the freely swimming zebrafish (Fig. 4), we acquired the baseline ECG signals prior to cryo-injury (Fig. 5a), and repeated the measurements at day 3 with a wearable MEA membrane secured to the chest. We observed that there were no significant drifts in ECG signals in terms of P waves and QRS complexes. Injury currents, namely ST-depression, persisted on day 3 (Fig. 5b). Unlike human ECGs, the T wave became more distinct in response to cryo-injury in zebrafish. Thus, the use of MEA membrane highlighted the feasibility of applying high-density electrodes to uncover small regions of myocardial ischemia with an minimally invasive approach [21].

c. Electrical signal acquisition of injury currents via multi-channels

In 4-chamber human hearts, 12-lead surface ECG has routinely been used to identify the specific sites of myocardial injury [21]. In the 2-chamber zebrafish heart, 4-lead surface ECG has made the detection of site-specific cryo-induced injury possible (Fig. 6b). Four working electrodes allowed for 4-lead ECG signal acquisition with improved spatial and temporal resolutions. Variations in the voltage amplitude among the four ECG signals reflected the different points of reference in electrode placement with respect to the cardiac conduction vector [4]. In this case, the P wave signals were more prominent in electrode leads B, C and D than that of A. Also notable were ST depression in electrode lead D, less significant ST depression in leads B and C, and absent ST depression in electrode lead A (Fig. 6c). Similar to the 12-lead ECG in humans, our 4-channel signal acquisition enabled detection of the specific sites where myocardial injury occurred for small animal models. In corollary, histology further corroborated the site-specific injury currents (Fig. 6a).

4. Discussions

The novelties of our study lie in the minimally invasive approach to uncover aberrant electrical phenotypes in injured myocardium for small animal models of heart regeneration. The multi-channel MEA membranes demonstrated, for the first time, the capability of detecting the site-specific ECG signals in the cryo-injured zebrafish hearts with high spatial and temporal resolution. The wearable MEA membrane remained functional in the freely swimming zebrafish, offering feasibility for long-term aquatic signal acquisitions. The integration of flexible and multi-channel approach further opens the avenue for scaling up to large vertebral animal model of tissue repair.

Heart failure remains the leading cause of morbidity and mortality in the US and developed world due to failure to adequately replace lost ventricular myocardium from ischemia-induced infarct. Studies of electrical phenotypes in small vertebral and mammalian heart regeneration using intimately implanted ECG microelectrode arrays provide new insights into electromechanical coupling of regenerating cells in host hearts. The advent of flexible microelectrode membrane allows for interrogation of electrical depolarization and repolarization. By interfacing microelectrode arrays with the injured and regenerating hearts, our strategy holds promises for long-term detection of arrhythmogenic signals in heart failure patients.

The wearable MEA membrane revealed the injured currents in the cryo-injured myocardium of both neonatal mouse and zebrafish hearts. While the MEA membrane was able to elucidate a distinct ST-depression (Figs. 3 and 5), site differences in micro-needle electrode placement reduced the sensitivity to detect the site-specific injured currents. In the neonatal mouse experiments, an extra needle electrode was used as a reference electrode in the MEA membranes to reduce the high impedance of the mouse skin. However, application of conductive epoxy to the surface of the builtin reference electrode would reduce the high impedance issue, improving SNR in ECG signals.

The MEA membrane was water-resistant (Fig. 4). Despite the rapid movement of zebrafish, the head portion of the MEA membrane remained anchored in the chest, allowing for reproducible P wave, QRS complex and T waves on day 3. In response to cryo-injury, there was a significant change in ECG signals (Fig. 5), revealing abnormal electrical repolarization in the cryo-injured heart. Currently, application of epoxy to the incision site of the fish promoted inflammatory responses, precluding further monitoring after day 3 (Fig. 2b). For future investigations, we propose to reduce the width of MEA at the incision sites and to develop an interlocking mechanism for securing the MEA without introducing stress to the animals. In another study, we have further demonstrated the evolving ECG signals 8 weeks after injury using our MEA membranes, and corroborated the previous observation that electrical repolarization or ST depression remained despite morphological evidence of heart regeneration at 2 months after injury [12] (Fig. S3a - sham and S3b - 8 weeks after injury).

Simultaneous 4-channel recording uncovered specific regions of injury currents with high spatial and temporal resolution. Surface ECG data from all four electrodes revealed

synchronization in ECG intervals (Fig. 6). The histology of injured myocardium (Fig. 6a) further corroborated the site-specific ECG signals. In this context, the SNR of our surface MEA is dependent on the placement of individual electrodes. In reference to detecting site-specific injury currents with high spatial resolution, the placement of electrode proximal to the injury site results in detecting ST depression. The electrodes distal to the injury site will not exhibit ST-depression. This electrophysiological phenomenon is in line with that of human surface ECG. We have overcome bioengineering challenges to address the small size, to match the Young's modulus between the flexible membrane and fish, and to obtain high signal-to-noise ratio (SNR) from the dynamic hearts. We have characterized the optimal electrode size of 300 μm in diameter for the best SNR. Further improvement in developing high-density MEA membranes would enable unequivocal detection of myocardial injury in the anterior, posterior and lateral walls of large animal models, similar to that of 12-lead ECG in humans.

The current MEA membranes also afford the feasibility of long-term electrophysiological monitoring. Our current approach is minimally invasive for the surface ECG acquisition. Alternatively, we can develop an epicardial approach by placing the MEA on the surface of the hearts. However, this approach is invasive, and the fish will be unlikely to survive from an open wound. A pre-clinical animal model such as miniature pigs would be a test-bed for placing higher density MEA via endocardial or epicardial approach for future investigations.

5. Conclusions

The wearable MEA membranes provide electrophysiological insights into the aberrant conduction phenotypes of regenerating tissues, otherwise difficult with the micro-needle approach. In comparison with the conventional needle micro-electrodes, the biocompatible and polymer-based MEA membranes demonstrated advantages in signal stability (Fig. 4), spatial resolution (Fig. 6), and minimally-invasive strategy. The micro-fabrication process is able to be integrated with CMOS technology for additional sensing modalities such as strain sensors for myocardial contraction. Our integration-enabled design paves the way for long-term water-proof electrical signal acquisition via wireless technology. As such, the polymer-based multi-channel MEA membranes hold promises for translational medicine from assessing ECG in cardiac repair to monitoring electroencephalography (EEG) in seizure or brain injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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1. The multi-channel MEA membranes demonstrate, for the first time, the capability of providing electrophysiological insights into the site-specific injury currents in the injured zebrafish hearts with high spatial and temporal resolution.
2. The MEA membrane remains functional in the freely swimming zebrafish, offering feasibility for long-term aquatic signal acquisitions.
3. The combination of water-resistant and wearable properties of MEA further allows for real-time assessment of electrophysiological signals in response to pharmacologic or genetic modifications without disturbing the animals.
4. The MEA technology can be integrated with wireless sensing technology for monitoring patients undergoing cardiac rehabilitation after heart attacks or to assess electroencephalography (EEG) signals after seizure and brain injury.

Fig. 1. Flexible MEA membranes for non-planar anatomy. (a) MEA membranes were dovetailed to the zebrafish anatomy. (b) The photo shows the EMA membrane flanked by flexible wings. (c) The photo revealed a flexible MEA membrane that could be conformed to the chest of neonatal mice.

Fig. 2.

Real-time acquisition of electrical conduction in zebrafish and neonatal mice. (a) The micro-electrode portion of the MEA membrane was implanted in the epicardium of zebrafish. A pair of transparent wings circumferentially enclosed the fish body. (b) The MEA membrane was implanted in a freely swimming zebrafish. (c) ECG acquisition in the Faraday cage illustrated the sedated zebrafish lying on a damped sponge. (d) Neonatal mouse ECG recording highlighted the MEA membrane adhered to the chest of the mouse and the external reference electrode positioned to the lower abdomen.

Fig. 3.

(a) Histological slides comparing sham and cryo-injured hearts. Aberrant electric phenotypes were acquired from neonatal mouse hearts 7 days post cryo-injury and sham operation. (b) Raw data was acquired via a MEA membrane on the chest. (c) Signal processing enhanced the SNR for ST-depression. (d) Validation was performed with micro-needle electrodes. Analogous to human rhythm recording with one lead, a single needle placement on the chest missed the ST-depression.

Fig. 4. Zebrafish ECG signals acquired via an implanted MEA membrane over 3 days. Electrical signal acquisition revealed P wave, and QRS complexes and T waves from the same animal on (a) Day 1, (b) Day 2, and (c) Day 3, respectively.

Fig. 5. ST depression in response to cryo-injury. ECG signals acquired from the implanted MEA membrane in the same zebrafish (a) prior to, and (b) post cryo-injury on day 3. Myocardial ischemia remained on Day 3 as evidenced by ST depression.

Fig. 6.

(a) Histological slides showing the evidence of cryoinjury. Simultaneous four-channel ECG signals were acquired. The implanted MEA membrane captured a region of myocardial injury otherwise undetected via the single electrode lead. (b) 4 channels were recorded simultaneously showing the synchronization in ECG intervals. (c) Electrode A revealed P, QRS, and T morphology without evidence of myocardial injury currents. Electrodes B, C and D (most obvious) uncovered a region of myocardium where cryo-injury induced ST depression.