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Flexible and waterproof micro-sensors to uncover zebrafish circadian rhythms: The next generation of cardiac monitoring for drug screening

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ABSTRACT

Flexible electronics are the next generation of sensors for mobile health and implantation. Zebrafish (*Danio rerio*) is an emergent strategy for pre-clinical drug development and toxicity testing. To address the confounding effects from sedation of fish and removal from the aquatic habitat for micro-electrocardiogram (μECG) measurements, we developed waterproof and wearable sensors to uncover the circadian variation in heart rate (HR) and heart rate variability (HRV) (Massin et al., 2000). The parylene-C based ECG sensor consisted of an ultra-soft silicone integrated jacket designed to wrap around the fish during swimming. The Young's modulus of this silicone jacket matched with the fish surface, and an extended parylene cable connected the underwater chest electrodes with the out-of water electronics. In addition, embedded micro-glass spheres in the silicone effectively reduced the effective density of the jacket to $\sim 1\ g\ cm^{-3}$. These innovations enabled physiological ECG telemetry in the fish's natural habitat without the need for sedation. Furthermore, a set of non-linear signal processing techniques filtered out the breathing and electromagnetic artifacts from the recorded signals. We observed a reduction in mean HR and an increase in HRV over 24 h at 10 dpa, accompanied by QT prolongation as well as diurnal variations, followed by normalization in mean HR and QT intervals at 26 days post ventricular amputation (dpa). We revealed Amiodarone-mediated QTc prolongation, HR reduction and HRV increase otherwise masked by sedation. The novel features of the flexible silicon jacket for μECG telemetry unraveled the biological clock and normalization of QT intervals at 26 dpa, providing the first evidence of new physiological phenomena during cardiac injury and repair as well as cardiac drug-mediated aberrant rhythms. Thus, the light weight and waterproof design holds promise to advance the next generation of mobile health and drug discovery.

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1. Introduction

The first generation of flexible electronics was established for micromachine-based shear stress sensor arrays mounted on the non-planar surface of air foils for turbulence control (Yong et al., 2003). Over the last decade, the advent of flexible microelectronic membranes is evidenced by the biomedical applications to interrogate electrical depolarization in the small vertebrate hearts (Ai et al., 2009; Ai et al., 2009), and the deployment of intravascular flexible shear stress sensors to assess atherosclerotic plaque (Ai et al., 2009). These parylene-based high-density electrode arrays have further enabled electrical stimulation in the retina to restore vision and spinal cord to restore locomotion (Rodger et al., 2007). Stretchable multi-electrode arrays (MEA) further unravel aberrant electrophysiological phenotypes of small animal models of heart regeneration (Cao et al., 2014). The MEA membranes adhere to the non-planar body surface, identifying spatial variations in cardiac injury currents from zebrafish hearts (Cao et al., 2014). The PDMS-based epidermal electronics revolutionized non-invasive monitoring for mapping cardiac conduction and brain activity (Viventi et al., 2010; Viventi et al., 2011). These high density arrays offer precise spatial control of stimulation and recording otherwise challenging with the traditional fine-wire electrodes (Rodger et al., 2007).

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Developmental genes involved in zebrafish heart repair are highly conserved in higher vertebrates. The average length of an adult fish is at 2–4 cm, accessible for relatively low-cost and high-throughput small molecule screening (Gibert et al., 2013). Their physiological complexity also provides conserved models of human disease for *in vivo* validation studies (Li et al., 2014). The biological characteristics of zebrafish are suitable for toxicity testing, including eco-toxicology (Zhang de et al., 2015). However, the *Clock* gene involved in the central oscillation to coordinate endogenous rhythms is linked to the generation of circadian rhythms (Whitmore et al., 1998). Thus, sedation of zebrafish influences the circadian variations in heart rate (HR) and heart rate variability (HRV) in response to cardiac injury or to drug testing.

The first micro-electrocardiogram (μECG) signals obtained from adult zebrafish required muscle paralysis (Milan et al., 2006; Yu et al., 2010; Lee et al., 2014). The gill motion was arrested to reduce electromagnetic (EMG) artifacts while oxygenation was provided to prevent hypoxia and arrhythmias via a needle-to-mouth resuscitation (Milan et al., 2006; Yu et al., 2010; Lee et al., 2014). Our group avoided paralytic agents to establish high signal-to-noise ratios for μECG signals via wavelet transform with Tricaine-based sedation (Yu et al., 2010; Lee et al., 2014). However, translating the zebrafish model to unequivocal drug screening and toxicity testing in the absence of sedation remained a challenge.

To address circadian rhythm-associated heart rates (HR) and heart rate variability (HRV), we have designed stretchable parylene cable and microelectrodes to establish 24 h telemetry of adult zebrafish (Massin et al., 2000). We performed real-time recording and analyses of μECG signals at 10, 18 and 26 days post ventricular amputation without sedation. We compared the nocturnal and daytime HR and HRV prior to and post ventricular amputation. We further identified changes in QTc intervals in response to Amiodarone, a class III anti-arrhythmic agent (Wu et al., 2008) in the presence versus absence of sedation. Our findings revealed HR and HRV in response to cardiac circadian rhythms, providing a physiological basis to advance drug development and

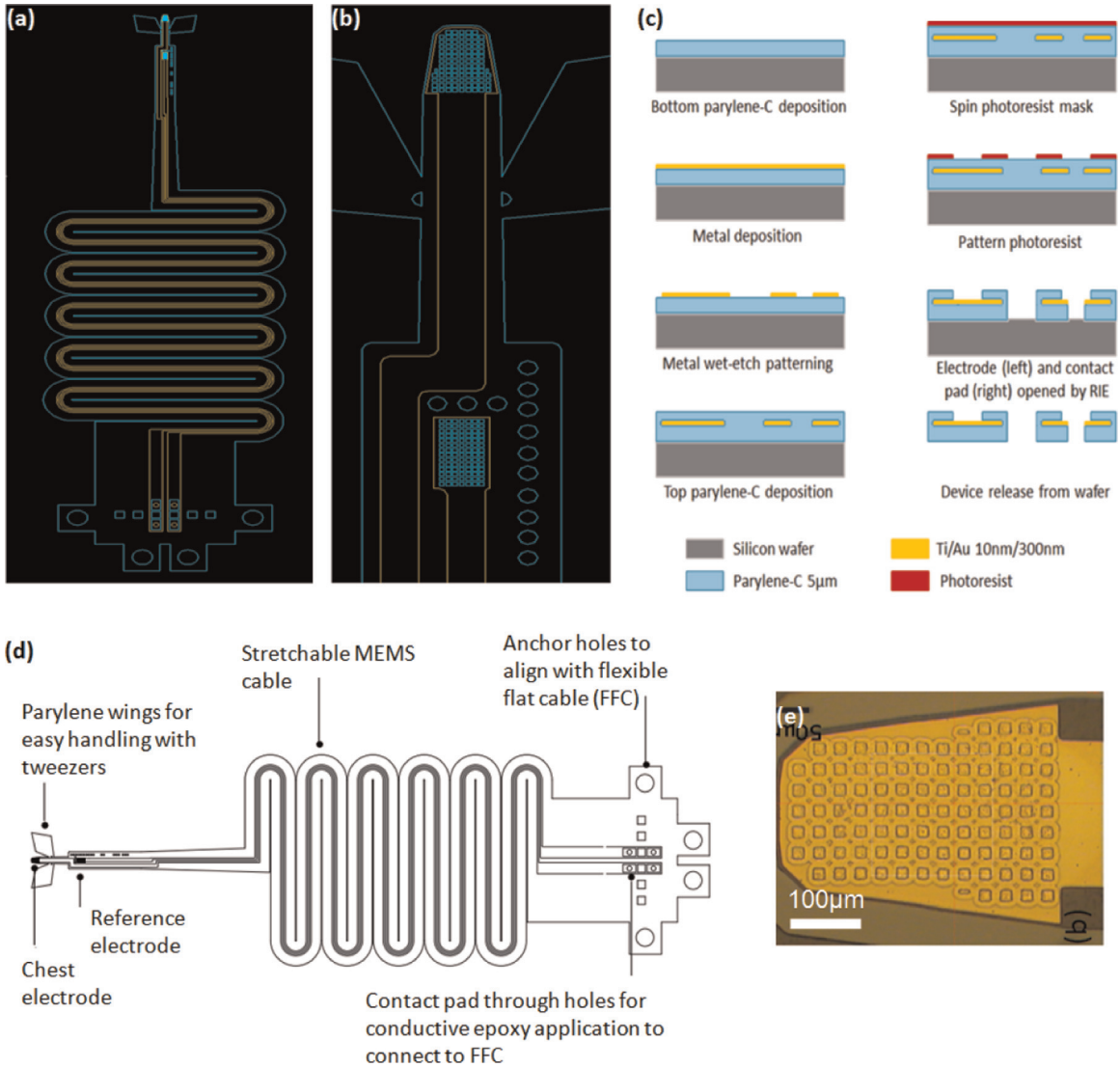

Fig. 1. Parylene C-based electrode and cable design, micro-fabrication, and device integration (a) The blue pattern illustrates the Parylene outline, and yellow pattern is the gold (Au) trace. Two Au electrodes (chest electrode and reference electrode) are patterned at 3 mm apart. Each electrode harbors a micro meshed structure with 5 μm × 25 μm openings designed to reduce stress on the Au thin film in response to stretching. The "wings" next to the chest electrode are designed to thread the zip tie ribbons into the silicone jacket, and are removed after the integration with jacket is complete. The PA holes around the reference electrode allow for anchoring the MEMS electrode/cable onto the jacket. Uncured silicone will be applied at these holes to adhere the electrode onto the jacket. (b) The zoomed-in view reveals the chest and reference electrodes. (c) Micro-fabrication process of the PA-Au/Ti-PA electrodes highlight the use of parylene C to embed electodes (d) Schematic diagram of the flexible cable and electrodes. (e) The zoomed-in view reveals the micro mesh openings in the chest electrode. Each opening is at 25 μm × 25 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)