CHRONOTROPIC BIOSENSING VIA STEM-CELL DERIVED MYOCYTE AGGREGATES

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Abstract-**Biosensors play a critical role in the chronotropic regulation of rate-adaptive electronic pacemakers. However, typical pacemaker biosensors only approximate physiological function via the measurement of surrogate signals such as ventilation, and therefore can be poorly correlated with chronotropic requirements. Alternatively, the electropotential input-output relationship of cardiac myocytes could be exploited for long-term, reversible quantification of chronotropic demand by monitoring the inherent rate effects of blood-borne catecholamines. Previously, we demonstrated the utility of this approach using murine whole-heart pinnal allograft transplants. Here, we advance this technique by utilizing pluripotent embryonic stem cell-derived cardiac myocyte aggregates implanted in the pinnae of syngeneic murine hosts. After one week, in all of the aggregates that showed sustained electropotential activity, there was $\geq$70% concordance between the myocyte-aggregate rate and endogenous heart rate over the course of the trial, thereby demonstrating the ability of the cell-based biosensors to sense humoral signals and track endogenous chronotropic dynamics. Improvements in myocyte-aggregate electropotential competency, along with further advancements such as catheter-based myocyte-aggregate systems, may facilitate the incorporation of such long-term, reversible biosensors into cardiac pacemakers or other devices that require humoral substance sensing.**

Keywords **- biosensor, stem cells, tissue engineering, pacemaker**

I. INTRODUCTION

One of the fundamental tasks required of implantable medical devices is accurate real-time determination of relevant functional physiological needs. For example, a cardiac pacemaker must determine the pacing rate required to supply the body with adequate cardiac output. Biosensors, which transduce biological actions or reactions into signals amenable to processing, are well suited for such monitoring. However, typical *in vivo* biosensors only approximate physiological function via the measurement of surrogate signals. Such surrogate-signal estimation is a prime source of error; e.g., cardiac pacemakers that use such signals often lack a high degree of dynamic fidelity with chronotropic requirements [1, 2].

A novel alternative approach is to use a biologically-based system that can sense physiological signals directly, thereby avoiding the approximation errors associated with surrogate-signal sensing. To this end we recently reported the development of such a tissue-based biosensor exploiting the endogenous signaling pathways of excitable tissue to couple the detection of *in vivo* physiological inputs to a functionally-responsive electropotential output [3]. Specifically, we studied the activity and regulation of remotely engrafted neonatal cardiac tissue in a murine model system. We found that the chronotropic dynamics of the exogenous excitable cardiac allografts were highly correlated with the activity of the endogenous heart. Moreover, pharmacological trials showed that the transplanted allografts were regulated by circulating catecholamines, suggesting that this approach may offer a foundation for the development of tissue-based biosensors for the detection of a range of blood-borne substances.

The present study was conducted to improve the functional biosensory utility of such excitable tissue-based biosensors. In particular, in an effort to translate such biologically-based biosensors into actual *in vivo* experimental or clinical tools, we developed sensors that utilize stem cell-derived myocyte aggregates in place of whole hearts. Such syngeneic, uniformly cultured aggregates would improve translational applicability and molecular plasticity, and therefore improve biosensor robustness and feasibility.

II. METHODOLOGY

A. Embryonic Stem Cell-Derived Cardiac Myocyte Transplant Model

Cardiac cell-based *in vivo* biosensors were developed with embryonic stem cell-derived cardiac myocytes in the place of whole neonatal cardiac tissue used in our previous study [3]. Spontaneously beating cardiac myocytes were derived from E9 murine pluripotent embryonic stem cells (American Tissue Culture Company, Rockville, MD) as previously described [4–6]. Briefly, embryonic stem cells were cultivated on a feeder-layer of primary mouse embryonic fibroblasts in DMEM culture medium supplemented with non-essential amino acids, L-glutamine, β-mercaptoethanol, 20% fetal calf serum, and 100 IU leukemia inhibiting factor (LIF). Droplets of cells (approximately 10^4 cells) in 30 μL of culture media without LIF were pipetted onto the lids of 3cm bacteriological petri dishes filled with phosphate-buffered saline (PBS) and cultivated for two days (at 37°C and 5% CO_2). The resulting aggregates were transferred from the hanging drops into 6cm dishes, fur-

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ther cultivated for five days, and then transferred to 12-well plates. Spontaneous chronotropic myocyte aggregates formed between 5 and 10 days after transfer and were subsequently employed in the murine pinnal transplant model. Specifically, the mice were pre-treated with platelet-derived growth factor (PDGF) (20 ng in 20 μL PBS). The following day, myocyte aggregates were physically dissociated and suspended in PBS (approximately 5×10^4 cells in 20 μL). These suspensions were transferred into the pinnal transplant pocket, which was then sealed via gentle pressure with forceps. Data acquisition for chronotropic activity assessment was performed 3-7 days post-transplantation as described below.

B. Electrocardiograms

Between 3 and 7 days post-transplantation, electrocardiogram (ECG) activity of the endogenous hearts and exogenous myocyte-aggregates were measured following intraperitoneal (IP) anesthetization with avertin. ECGs were acquired for approximately 60 min via an A-M Systems Model 1700 four-channel differential AC amplifier. Signals were bandpass filtered between 3.0 and 100.0 Hz, notch-filtered at 60.0 Hz, amplified 1000X, and sampled at 500 Hz by a National Instruments AT-MIO-16E-10 data acquisition board on a 266 MHz Intel Pentium-II computer running Real-Time Linux [7]. Transplant chronotropic activity was defined by two criteria. "Sustained" activity was characterized by consistent, monomorphic, periodic waveforms that continued for at least 200 seconds. "Sporadic" activity was characterized by a range of activity including short-lived, irregular, multimorphic activity, regular activity lasting less than 200 seconds, and slow, scattered monomorphic waveforms that recurred multiple times throughout the recording period.

C. Quantitative Rate Analysis

Post-acquisition automatic (with manual correction as needed) ECG excitation annotation was performed using custom Linux C++ software. Excitations were defined as the R-waves for the endogenous hearts and the aggregate action potentials for the myocyte aggregates. Mean inter-excitation intervals RR were computed every two seconds so that the dynamics of the endogenous and exogenous signals, which have different inherent rates, could be compared quantitatively at synchronized time slices.

D. Endogenous-Exogenous Cardiac Chronotropic Correlation

Recordings from the exogenous and endogenous tissue were analyzed for "relative" (the ability of the exogenous myocyte aggregate to sense increasing and decreasing endogenous heart-rate trends) and "absolute" (the ability to sense absolute heart rate; i.e., one-to-one correspondence) chronotropic tracking. Discrete data sets of at least 200 s were fit (using Matlab 5.3.1) to a continuous-time polynomial function $RR(t)$ as previously described [3]. The concordance of the endogenous and exogenous signals was computed as the fraction of the time that their derivatives $dRR(t)/dt$ [computed analytically from the fitted polynomial function $RR(t)$] had the same sign. A concordance of ≥ 0.70 was employed as a measure of the ability of the exogenous myocyte aggregates to track the increases and decreases in endogenous rate. Absolute chronotropic correlation was measured by the correlation coefficient computed between each exogenous and corresponding endogenous RR time series [3].

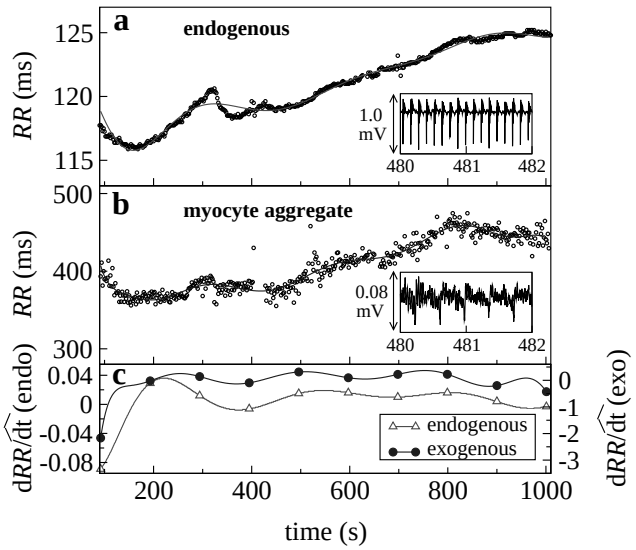

Fig. 1: Representative example of the mean inter-excitation intervals RR versus time for the endogenous heart **(a)** and the ES-cardiac myocyte transplant **(b)** of a mouse pre-treated with PDGF. The insets in panels **(a)** and **(b)** show segments of the endogenous and ES-cardiac myocyte electrocardiograms, respectively. The insets show that excitations were in the negative-voltage direction for both recordings in this trial. First order derivatives versus time $dRR(t)/dt$ of the polynomial fits of the RR dynamics **(c)** demonstrated an 80% concordance in sign for the trial, and therefore a high degree of "relative" sensing ability.

III. RESULTS

The majority (30/37) of the myocyte-aggregate transplants demonstrated spontaneous or sustained electropotential activity after one week. Moreover, 6/30 of the electrically viable cellular transplants demonstrated sustained depolarizations. Importantly, all of the sustained-activity myocyte aggregates acted as relative biosensors of the endogenous chronotropic dynamics (i.e., with $r \geq 0.70$). Figure 1 shows one such relative tracking example. Furthermore, as shown in the example of Figure 2, the myocyte aggregates showed a high degree of absolute tracking; for the 6 sustained-activity aggregates, the correlation coefficients between the myocyte-aggregate RR and the endogenous RR were $r = 0.80 \pm 0.15$ (mean $\pm$ standard deviation). Such absolute sensing ability suggests that signals from such biosensors could be employed as direct indicators of endogenous heart rate.

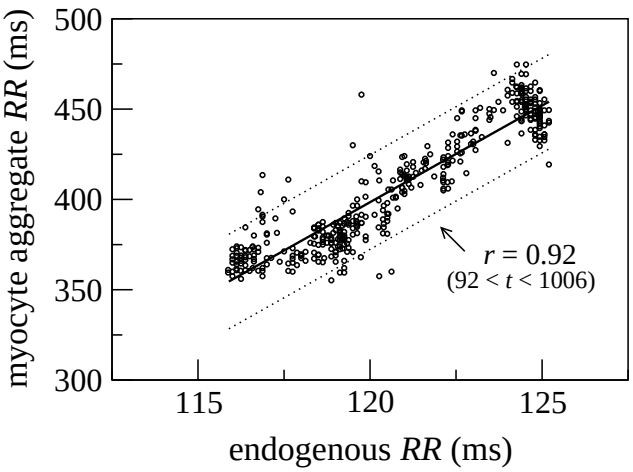

Fig. 2: Representative example of ES-cardiac myocyte-aggregate RR versus endogenous RR in a mouse pre-treated with PDGF. For this trial, the correlation coefficient $r = 0.92$, indicating a high degree of "absolute" tracking ability.

IV. Discussion

In this study we have extended the development of a new class of biologically-based biosensors that exploit the electropotential input-output relationship of cardiac myocytes for long-term, reversible quantification of blood-borne substances. Specifically, we have demonstrated the ability of transplanted pluripotent embryonic stem cell-derived cardiac myocyte aggregates to function as effective sensors of endogenous heart-rate dynamics.

One potential application of such biologically-based biosensors is to serve as the chronotropic sensing element for implantable cardiac pacemakers. By utilizing the inherent ability of cardiac myocytes to regulate chronotropy by setting electronic pacing rate according to sensed humoral signals, such a pacemaker would avoid the approximation errors associated with the surrogate-signal rate estimates utilized by current rate-adaptive pacemakers.

We project that the utility of this approach will extend beyond that of chronotropic regulation biosensing. Molecular engineering may offer a means for the detection of physiological and pathophysiological signals that do not routinely alter cardiac chronotropy. Indeed, excitable-cell biosensor systems could lead to the development of long-term, physiologically-tuned, functionally integrated bioprocessing interfacing with a range of external or implantable devices to facilitate the rapid initiation of appropriate actions.

We recognize that furthering the feasibility of cardiac myocyte-based biosensors will require that the cardiac myocytes be derived from syngeneic sources of stem cells such as the bone marrow. Recent murine studies have demonstrated that cardiac myocytes can be derived from bone marrow cells [8]. This approach may allow for the potential clinical translation of cell-based chronotropic biosensor systems. Further advances in *in vivo* biosensors might employ such cells on silicon chips or other defined biocompatible materials [9–11].

In short, the employment of genetically plastic stem-cell technology to detect blood-borne signals should facilitate the development and potential clinical translation of this approach for the direct biological detection of physiological and pathophysiological signals. This may one day lead to the incorporation of such long-term, reversible biosensors into cardiac pacemakers or other devices that require humoral substance sensing.

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