A WIRELESS PASSIVE STIMULATOR OF CARDIOMYOCYTES

Shiyi Liu¹, Ali Navaei², Mehdi Nikkhah² and Junseok Chae¹

¹School of Electrical, Computer and Energy Engineering, Arizona State University, USA

²School of Biological and Health System Engineering, Arizona State University, USA

ABSTRACT

Electrical stimulation to promote cardiac cell function, is well established; yet current methods are all limited to wired stimulation systems. To overcome this limitation, herein we report a wireless passive stimulator to induce contraction in cardiomyocytes with a desired frequency. The wireless stimulator contains passive electrical components, on-board antenna, and one pMOS, and is activated by external RF carrier/pulses. The performance of the stimulator is evaluated on neonatal rat cardiomyocytes, side by side by wired stimulation. The results show that synchronous cell beating behavior via wireless stimulation concurs well with those upon wired counterparts, suggesting that the wireless passive stimulator holds significant potential in heart electrical stimulation and regeneration.

INTRODUCTION

Applying electrical pulse stimulation on the cardiac cells, to mimic the bio-potential signal generated in native heart, has been proven to significantly improve the key properties of the engineered tissues such as cell alignment, protein expression, and contractility [1]. Currently, the electrical stimulation is mainly executed *in vitro* when cardiac tissue is exposed to electrical signal source, such as a function generator. Applying electrical stimulation *in vivo* under an implanted environment holds a substantial challenge mainly due to the wiring setup, imposing severe limitations on regeneration and repair of damaged heart. Despite numerous attempts on wireless implantable stimulator [2-4], few have been specifically designed to stimulate cardiac cells and produces comparable results to a wired counterpart. Here we report a wireless passive cardiac cells stimulator, which features battery-free, little power consumption, and nearly zero heat generation. The stimulator successfully delivered stimulation pulses large enough to induce synchronized beating behavior of cardiomyocytes, suggesting a promising application on heart repair and regeneration.

Figure 1(a, b) shows the structure of the passive stimulator prototype, which consists of a plastic substrate, an on-board antenna, and multi-stage charging/discharging circuits for generating electrical pulse stimulation. The antenna is designed and simulated in HFSS to optimize its directivity and radiation efficiency. Frequency of the RF signal is chosen to be 2.4 GHz based on previous published work [5]. Output of the antenna is fed to the circuits shown in figure 1(c). The principle of the circuits is to use charge pump to increase voltage level and store charges on output capacitor C_4 . The pMOS T_1 is used to control charging/discharging of C_4 to generate output pulse.

One unique characteristic of the stimulator which distinguishes it from previously reported wireless

stimulators [3-4], is the onboard antenna to establish RF communication between the stimulator and external system, compared to utilizing inductive coupling. The inductive coupling imposes a challenge of requiring large cross-sectional orthogonal planes between the external primary and internal secondary coils, which not only causes inefficient power transfer in implantable environments but also may induce unwanted current on the tissue [2-3]. The designed wireless stimulator consumes little power, thus facilitates the RF coupling. When the stimulator is at rest, T_1 turns “off”. The equivalent circuits in this state consist only of diodes and capacitors. Charges are stored at the output capacitor, C_4 , and ideally no power consumption exists during this process. When T_1 turns “on”, the charges accumulated on C_4 begin to discharge, generating a pulse signal across the load. Again, due to the very short pulse duration (msec), the stimulator consumes low power during this process. As a result, total power consumption is extremely low which allows RF coupling to drive the stimulator. The low power consumption also ensures the safety of the stimulator as an implant, via little heat generation *in situ*.

Figure 1(b) shows the photograph of a fabricated wireless stimulator on Acrylic. The antenna and routing of circuits were formed by a thin gold film (Cr/Au; 20/100 nm). Discrete electronic components were mounted using conductive silver epoxy (Electron Microscopy Science). The preliminary proof-of-concept stimulator sizes $50 \times 60 \text{ mm}^2$. Once we verify the functionality of the stimulator, we will miniaturize it by decreasing the footprint of antenna and implementing circuit integration techniques.

Figure 1: (a). Schematic of the wireless passive stimulator; (b) Photograph of a fabricated stimulator on acrylic sheet; (c) Circuit diagram of the stimulator.

Figure 2 illustrates the external system for RF modulating and transmitting. We use an arbitrary wave generator (Agilent 33250A), to produce a reverse pulse to

AM modulate the 2.4GHz RF carrier, which is generated by RF signal generator (Agilent E4432B). The modulated signal is amplified and radiated through an external antenna (A10194, Antenna). The stimulator receives and demodulates the modulated signal to generate output pulses where frequency and pulse width are determined by the modulated input.

Figure 2: Schematic of external RF modulation.

EXPERIMENT SETUP AND RESULTS

We conducted electrical stimulation in a custom made bioreactor chamber (figure 3(a)) which was built based on previously established protocols [6]. Briefly, two parallel carbon electrodes (5 mm length) were placed on a plastic petri dish (6 mm diameter) with 1 cm spacing. The carbon electrodes were fixed using silicon adhesive. Platinum wires were attached to each of the carbon electrodes at the opposite ends and were fully covered by silicon adhesive. After assembling, we filled the bioreactor with Dulbecco's phosphate buffered saline (DPBS) and used it as the load of the wireless stimulator. The applied electrical signal was a 1 Hz, monophasic square-wave pulse with 2 ms pulse duration. This parameter was also chosen based on protocol in order to mimic those existed in nature [1].

The electrical property of human tissue is known to be capacitive rather than purely resistive [7]. For RF powered implanted wireless devices, the capacitance imposes loading on the output signal. The loading effect needs to be characterized, especially when the wireless devices often have finite power delivery capability. Therefore, before using bioreactor, we characterized the output of the wireless stimulator with various loading capacitors as shown in figure 3(b). As the loading capacitors become larger, amplitudes begin to drop due to output power limitation. However, even when the loading capacitance is as high as several microfarads, the amplitude can rise over 5 V, which is sufficient for stimulating cardiac cells.

Using bioreactor as the load, we characterized the output amplitude of the wireless stimulator with both radiated RF power and wireless transmission distance. Figure 3(c) displays the measured pulse amplitude changes as a function of RF power at various distances. It can be seen that amplitude drops quickly as RF power decreases or the distance increases. The radiated RF power was limited to 30 dbm (1 W) according to FCC regulations [8]. As a result, the wireless stimulator was required to be placed

within 5 mm away from the external antenna to produce sufficient voltage output for cardiac cells.

Figure 3: (a). Photograph of bioreactor which the wireless passive stimulator was evaluated. (b). Amplitude of the electrical pulse output as a function of loading capacitance. (c). Amplitude of the electrical pulse output to the bioreactor as a function of RF power at different RF transmission distances.

We obtained ventricular cardiomyocytes from 2-day old neonatal rats based on previously established protocols approved by Institution of Animal Care at Arizona State University [9]. Briefly, heart was surgically cut from the thorax and was sliced into pieces and placed inside trypsin solution prepared in Hank's balanced salt solution for 14-16 hours. Then, heart pieces were neutralized and subjected to collagenase treatment to release the cell populations. Gelatin methacrylate (GelMA) hydrogel was synthesized as following [9]: first, type A gelatin (Sigma-Adrich) (10% w/v) was fully dissolved in DPBS at 50°C. Next, methacrylate (MA) (8% v/v) was added dropwise to the gelatin solution and stirred for 3 hours at 50°C. The solution was diluted five times to stop methacrylation reaction. Then, the solution was placed in dialysis tubes (12-14 KDa molecular weight cutoff), which was placed in deionized water at 45°C under stirring for 7 days to eliminate unreacted MA and salt. The dialyzed solution was filtered using a 0.2 μ m membrane and then freeze-dried for 7 days to obtain GelMA foam. Gold nanorods (GNRs) were also synthesized via a seed-mediated silver-assisted growth method [10]. Briefly, gold nanoparticles (~4 nm diameter) were synthesized and used as seeds. Next, the seeds were grown anisotropically in growth solutions containing silver nitrate (AgNO_3) and ascorbic acid. Finally, gold nanorods were separated using high-speed centrifuge at 14000 rpm for 12 min. GelMA-GNR hybrid hydrogel constructs (150 μ m thick) were fabricated by photo-crosslinking of GelMA-GNR prepolymers solution using UV light (25 sec exposure time). Isolated cardiomyocytes (750,000 cells/sample) were then seeded on GelMA-GNR hybrid hydrogels and fed by cardiac culture media. The media was exchanged with fresh one every other day. Cardiac tissue constructs were utilized

for external stimulation on day 8 of culture.

Cardiac tissue constructs (figure 4(a)) were placed between the two carbon electrodes of the bioreactor, and the bioreactor was placed under microscope (Observer Z1, Zeiss) to monitor cell contraction activity. Once synchronous contractility was observed, a video was captured. At the same time, electrical signal output from stimulator was recorded using data acquisition card (NI6216, National Instrument) and NI signal Express. Electrical current output signal was obtained by measuring the voltage drop across a $1\ \Omega$ resistor that was connected in series with the bioreactor. After real time cell contraction recording, we utilized a custom-written MATLAB code to process the videos and generate representative cell beating signals. We conducted both wired and wireless stimulations, and an arbitrary waveform generator (BK Precision 4052) was used as control to apply wired electrical stimulation.

Figure 4: (a) Representative phase-contrast image of cardiac cell; (b) Cardiac cells beating activity at 0.5, 1, 2Hz frequencies under wired and wireless stimulations.

Figure 4(b) shows the representative cell beating signals obtained at 0.5, 1, and 2Hz frequencies for wired and wireless stimulations. Little discrepancy exists between cell beating curves under wired and wireless stimulation, suggesting the robust functionalities of the wireless stimulator. This is the first attempt, as far as the authors are aware, to stimulate synchronous cardiac cell contractions using a wireless stimulator.

To further evaluate the performance of the wireless stimulator, we recorded the output voltage and current waveform from wired/wireless stimulators (figure 5) during cells contractions. Relatively high current (40-60 mA) was needed to successfully stimulate the cardiac cells. The high current driving capability is beyond the output capability of previously reported wireless stimulators [2-4]. The wireless

stimulator shows a rapid drop of current and voltage profiles. This phenomenon is caused by the voltage discharging behavior of the onboard capacitor. The output voltage and current profiles of wired stimulator (function generator) showed much less change, as expected, because the function generator has significantly larger driving capabilities than the wireless stimulator.

Figure 5: Electrical voltage and current output applied to cardiac cell recorded in (a) wired stimulator and (b) wireless stimulator.

The wired stimulator is a commercialized function generator whose output resistance is fixed at $50\ \Omega$ in order to match $50\ \Omega$ transmission line cable. On the other hand, wireless stimulator has an output resistance as high as $10\ \text{k}\Omega$. The difference between output resistances directly impacts the discharging speed of any charges stored in bioreactor. As mentioned in published study [1], bioreactor is highly capacitive at DC due to the electrolytes inside. For the wireless stimulator, the combination of large capacitance of bioreactor and high resistance of stimulator resulted in a long discharging time, leading to the non-zero low level waveform shown in figure 5(a). Although the output waveform from wireless stimulator demonstrates inconsistency with its wired counterparts, it is sufficient to induce well controlled cells contractions, achieving the goal of electrical stimulation.

Figure 6: Comparison of voltage excitation thresholds of wireless and wired system 0.5, 1, and 2 Hz frequencies.

Figure 6 illustrates the measured voltage excitation thresholds for wired and wireless stimulators. Excitation threshold is defined as the minimum voltage required for cardiac cells to start beating synchronously. As the frequency becomes higher, the excitation threshold increases, suggesting it become difficult to induce synchronized contraction of the cardiomyocytes. This result corresponds well with the prior studies [1]. At 0.5 and 1 Hz, no significant difference was observed within the measured

average excitation thresholds for wired and wireless stimulation (around 3 V). Yet at 2 Hz, the excitation threshold for wireless stimulation became 5V, which is slightly larger than that of wired stimulation (3.5V). This increase might be due to the decrease of pulse width which is shown in figure 5.

Here we reported a wireless passive stimulator to control of the synchronized beating behavior of the GelMA-GNR hybrid hydrogel tissue constructs. The fabricated stimulator is a preliminary proof-of-concept of the prototype, and the footprint and operating distance need to be improved in the future.

The onboard antenna currently occupies large area. This is because of the performance of antenna is a strong function of its electrical length. As the antenna becomes smaller, many of its important characteristics decrease, such as radiation resistance, efficiency, and directivity. Therefore, designing a small size antenna with acceptable performance requires many trade-offs. We believe it is possible to drive the presented wireless stimulator with an antenna that is small enough to be injected. This is due to the fact that very little power is consumed by the stimulator itself, mitigating the design requirement for antenna power output capability.

In this work, all measurements were performed in the air at a very close wireless transmission distance. In the real implanted setting, EM wave keeps attenuated as they propagate within the body tissues. This makes it even more difficult for stimulator to generate sufficient voltage signal *in vivo*. These all demand the stimulator needs to be improved in practical settings. Several mitigations exist, including optimized multi-stage charging/discharging circuits, directivity enhancement of the antenna, and minimization of RF loss in the plastic substrate. Evaluating the wireless stimulator in a tissue emulating phantom also conveys more realistic performance evaluation, which remains as our future study.

The wireless passive stimulator may find potential applications in cardiac stimulation and regenerative therapy [11], through integration/injection of engineered tissues/cells. Currently, the success of this therapy remains hindered by low survival rate and unsynchronized contractions of the transplanted cells [11]. We believe that by injecting a wireless passive stimulator, it would be possible to synchronize cardiac contraction, thus improving the effect of cardiac regenerative therapies.

CONCLUSION

In this work, we report a wireless passive stimulator for inducing of contractions of engineered cardiac tissues. The cardiac cells beating signals recorded under wired/wireless stimulation show little discrepancy. Recorded output electrical signals from the stimulator demonstrate output current amplitude larger than 60 mA, which is significantly higher than those of previously reported stimulators. Cell excitation thresholds for wired/wireless stimulation were measured and compared, which revealed proper agreement with recorded electrical signals. Further optimization and

miniaturization of the wireless passive stimulator hold great potential in cardiac regenerative therapy.

ACKNOWLEDGMENTS

This work is partially supported by National Science Foundation (#1344928).

REFERENCE

- [1] N. Tandon, C. Cannizzaro, P. G. Chao, R. Maidhof, A. Marsano, H. T. H. Au, M. Radisic, and G. Vunjak-Novakovic, "Electrical Stimulation Systems for Cardiac Tissue Engineering", *Nature Protocols*, 4, 155-173 (2009).
- [2] P. J. Larson and B. C. Towe, "Miniature Ultrasonically Powered Wireless Nerve Cuff Stimulator", *Proceedings of the 5th International IEEE EMBS Conference on Neural Engineering*, Cancun, Mexico, April 27, 2011, pp. 265-268.
- [3] B. Ziaie, M. D. Nardin, A. R. Coghlan, and K. Najafi, "A Single-Channel Implantable Microstimulator for Functional Neuromuscular Stimulation", *IEEE Transactions on Biomedical Engineering*, 44, 909-920 (1997).
- [4] H. M. Lee, H. Park, and M. Ghovanloo, "A Power-Efficient Wireless System With Adaptive Supply Control for Deep Brain Stimulation", *IEEE Journal of Solid-State Circuits*, 48, 2203-2216 (2013).
- [5] A. Abbaspour-Tamijani, M. Farooqui, B.C. Towe, and J. Chae, "A miniature fully-passive microwave back-scattering device for short-range telemetry of neural potentials," in *Proceeding of 30th Annual International IEEE/EMBS Conference*, August 2008, pp. 129-132
- [6] A. Navaei, D. Truong, J. Heffernan, J. Cutts, D. Brafman, R. W. Sirianni, B. Vernon, and M. Nikkhah, "PINPAAM Based Biohybrid Injectable Hydrogel for Cardiac Tissue engineering", *Acta Biomaterialia*, 32, 10-23 (2016).
- [7] H. P. Schwan and C. F. Kay, "Capacitive Properties of Body Tissues", *Circulation Research*, 5, 439-443 (1957).
- [8] Federal Communication Commission, Code of Federal Regulations, Title 47: Telecommunications, Part 15: Radio Frequency Devices, 2007.
- [9] H. Saini, A. Navaei, A. V. Putten, and M. Nikkhah, "3D Cardiac Microtissues Encapsulated with the Co-Culture of Cardiomyocytes and Cardiac Fibroblasts", *Advanced Healthcare Materials*, 4, 1961-1971 (2015).
- [10] B. Nikoobakht and M. A. El-Sayed, "Preparation and Growth Mechanism of Gold Nanorods (NRs) Using Seed-Mediated Growth Method", *Chemistry of Materials*, 15, 1957-1962 (2003).
- [11] G. Vunjak-Novakovic, K. O. Lui, N. Tandon, and K. R. Chien, "Bioengineering Heart Muscle: A Paradigm for Regenerative Medicine", *Annual Review of Biomedical Engineering*, 13, 245-267 (2011).

CONTACT

* Shiyi Liu, tel: +1-480-758-2753; Shiyi.Liu.1@asu.edu