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Design of Electrical Stimulation Bioreactors for Cardiac Tissue Engineering

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Abstract— **Electrical stimulation has been shown to improve functional assembly of cardiomyocytes in vitro for cardiac tissue engineering. Carbon electrodes were found in past studies to have the best current injection characteristics. The goal of this study was to develop rational experimental design principles for the electrodes and stimulation regime, in particular electrode configuration, electrode ageing, and stimulation amplitude. Carbon rod electrodes were compared via electrochemical impedance spectroscopy (EIS) and we identified a safety range of 0 to 8 V/cm by comparing excitation thresholds and maximum capture rates for neonatal rat cardiomyocytes cultured with electrical stimulation. We conclude with recommendations for studies involving carbon electrodes for cardiac tissue engineering.**

I. INTRODUCTION

A biomimetic approach to cardiac tissue engineering may be designed to recapitulate any number of aspects of the *in vivo* environment, including 3-dimensional structure and/or biochemical cues [1], physical forces (electrical [2] and/or mechanical [3][4]). Our overall approach includes physiologic density of cell subpopulations in a 3D setting (to enable cell communication and coupling), convective-diffusive oxygen supply by medium perfusion through channeled scaffolds (to mimic the role of capillary network), supplementation of oxygen carriers (to mimic the role of hemoglobin), and induction of macroscopic synchronous contractions of cultured constructs by electrical signals designed to mimic those in native heart [2]. After only 8 days in vitro, such stimulation resulted in increased amplitude of synchronous construct contractions and in a remarkable level of ultrastructural organization [5]. We focus here on the electrical stimulation component of our model system.

Stimulation efficiency is determined by the ability to attain the desired physiological response (improving functional assembly of cardiomyocytes in vitro) with minimal damage to the surrounding tissue. This issue is of particular importance to us because, in a electrochemical system such as the one described here (Fig. 1), charge transfer can occur through three mechanisms: (i) non-faradaic charging/discharging of the electrochemical double layer, (ii) reversible faradaic reactions, and (iii) non-reversible faradaic reactions [6]. The first two mechanisms are desirable, while the last should be avoided because it is associated with electrode degradation and harmful byproducts. In past studies, we have determined carbon electrodes to be the electrode of choice due to their passive biocompatibility, high availability, low price, superior charge injection characteristics and high resistance to chemical reactions and corrosion [7][8]. In the present study we aim to develop rational experimental design principles in terms of both bioreactor configuration (varying electrode configuration, input voltage, and age) and effects of varying stimulation amplitudes during culture on electrical excitability of engineered cardiac tissue.

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II. METHODS

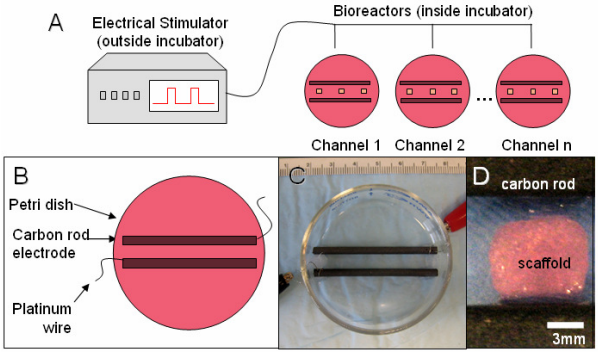

Fig. 1. Experimental setup for applying pulsatile electric field stimuli to cardiac cells. (A) Overview of experimental setup. An electrical stimulator generates the pulses which are transmitted to bioreactors located inside an incubator maintained at 37° C. (B) Schematic diagram of the electrical stimulation chamber (modified 60 mm Petri dish with carbon rod electrodes). 3-D scaffolds may be placed in between the electrodes. (C) Photograph of assembled electrical stimulation chamber. (D) Close up view of scaffold positioned between electrodes.

The effects of electrode configurations, different input voltages, and ageing were assessed, in that order, before any cell studies were performed. After performing characterization of various electrode configurations compatible with Petri dishes 100 mm in diameter and smaller (to be compatible with standard cell culture practices), we chose a single configuration which seemed not only to be a good candidate in terms of electrical performance, but would match our bioreactor needs. All subsequent studies (input voltage, current injection and cell studies) were performed with this configuration.

A. *Bioreactor Configuration*

The effects of electrode configurations, different input voltages, and ageing have all been assessed by performing current measurements and by using electrochemical impedance spectroscopy (EIS) as previously described [7][8][9]. In short, EIS measurements were taken with an electrochemical interface (Solartron 1287) and a frequency response analyzer (FRA, Solartron 1250) controlled by a computer with ZPlot software. EIS spectra were acquired for each electrode over a frequency range from 1 x 10^{-2} Hz to 1 x 10^{6}, with perturbation amplitudes (input voltages) ranging from 10 mV to 3V. Collected data were evaluated in ZView 2.5b software to generate Nyquist and Bode plots, and from these, data were fit to a simple "Randles' cell" equivalent circuit (Fig. 2) using instant fit functions.

In a Randles' cell, R_s represents the resistance of the bulk solution, R_p represents the polarization resistance (the electrode's resistance to corrosion), and $C_{double\text{-}layer}$ represents the capacitance of the double layer at the electrode/electrolyte interface. Due to non-homogeneities in electrochemical systems, we use a so-called "constant-phase element" (CPE) in lieu of a capacitor [10][11]. A CPE's impedance is of the form $Z = Z_0/(j\omega)^{\eta}$, where the factor η expresses the degree of non-ideality of the CPE, and ranges in value from 0 to 1. For an ideal capacitor, η is equal to 1, but decreases with increasing "non-ideality."

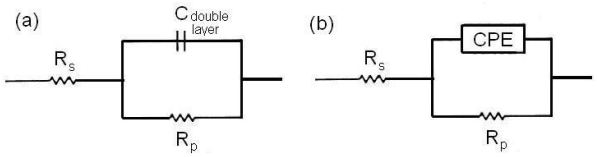

Figure 2: (a) The equivalent circuit of a simple electrochemical "Randles" cell, where R_s represents the solution's resistance, $C_{double\ layer}$. the double layer capacitance, and R_p the polarization resistance. (b) The equivalent circuit of a simple electrochemical cell with a CPE instead of a double-layer capacitance. This equivalent circuit is generally a more accurate description of electrochemical systems.

B. *Cardiac Cell Culture with Electrical Stimulation*

Cardiac constructs were prepared as previously described [2][5][7] by seeding collagen sponges (6 mm x 8 mm x 1.5 mm) with cell populations isolated from neonatal rat heart ventricles (6 million cells per scaffold) and stimulated (while maintained in a constant position with respect to the electrical field gradient) for 5 days using square monophasic pulses (2 ms duration, 1 Hz, 0-12.5 V/cm) after three days of preculture without electrical stimulation. Cells were cultured in high-glucose DMEM + 10% FBS at all times. Contractile activity was assessed visually with stereomicroscopy while pacing cardiac constructs, and measuring excitation threshold (ET), and maximum capture rate (MCR) as previously described [7][9].

II. RESULTS

A. *Bioreactor Configuration*

When we compare R_s values measured for different configurations (measured with an input voltage of 10 mV) to calculated values for a parallel-plate linear model of R_s:

$$R = \frac{d}{\sigma A} \tag{1}$$

where R is resistance, d is the distance between the electrodes, σ is the conductivity of the solution, and A is the surface area exposed to the electrolyte (Fig. 3A), we see that

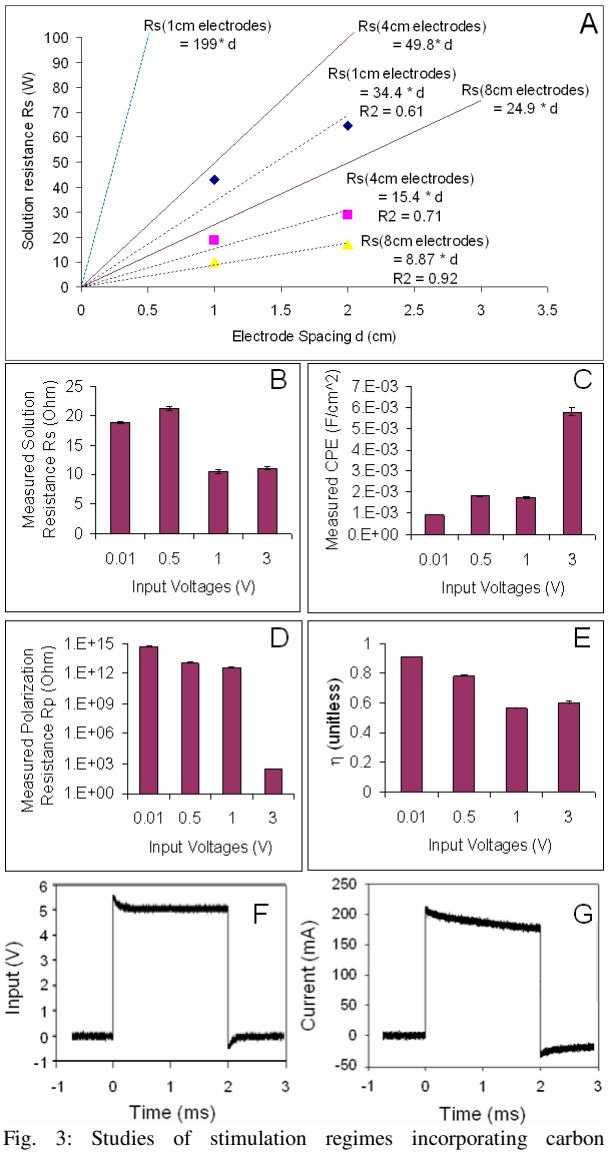

Fig. 3: Studies of stimulation regimes incorporating carbon electrodes. (A) Measured and theoretical values for solution resistance at various electrode configurations, with corresponding linear best-fit lines. (B) Decreasing measured solution resistance at input voltages ranging from 10 mV-3 V. (C) Increasing measured CPE values at input voltages ranging from 10 mV-3 V. (D) Decreasing measured polarization resistance values at input voltages ranging from 10 mV-3 V. (E) Decreasing measured η values at input voltages ranging from 10 mV-3 V.

for increasing electrode length, the data more closely fits the functional form of eqn 1 as assessed by the R^2 value of the linear fits. Moreover, as the electrode length increases the experimental values for the slopes $1/\sigma A$ more closely approximate the theoretical values, as evidenced by the fact that the ratio between experimental and theoretical $1/\sigma A$ decreases from 5.8 for 1 cm electrodes, to 3.2 for 4 cm electrodes, to 2.8 for 8 cm electrodes. Given that our scaffolds are 6 mm x 8 mm x 1.5 mm in size, and that 4-cm long and 8-cm long configurations (with respect to theoretical values) are equivalently similar to theory, we chose 4-cm long electrodes with 1-cm spacing for all further studies reported here.

When comparing electrode behavior with increasing input voltages but under conditions of constant geometry, we see that all calculated equivalent circuit parameters change (Fig. 3B-E). More specifically, with increasing input voltage, solution resistance, polarization resistance and η all decrease, while CPE increases.

By applying a typical pulse to the bioreactor (Fig. 3F) and examining the current flowing through it (Fig. 3G) we can determine that charge is not only transduced to the cells during the 2 ms pulse (0-2ms) but also that the vast majority of injected charge (approximately 85%) is transmitted to the culture medium via reversible processes. In addition, when we compare new electrodes to those used for just one experiment (Table I), although polarization resistance does not change appreciably (as previously shown [7]), we see a decrease in R_s, an increase in CPE and a decrease in η values.

TABLE I
BEFORE AND AFTER ONE EXPERIMENT

Age	Rs(Ω) (% error)	CPE (F) (% error)	Rs(Ω) (% error)	η (% error)
New	16.15 (1.38%)	7.68E-4 (1.67%)	3.59E+13 (0.18%)	0.91 (0.60%)
Aged	14.90 (1.47%)	2.0E-3 (1.90%)	1.37E+13) (0.35%)	0.87 (0.77%)

Ω = Ohm, F =Farad

B. Cardiac Cell Culture with Electrical Stimulation

By examining excitation threshold (ET) of the engineered constructs accompanying increasing levels of electrical stimulation during culture (Fig. 4A), we can observe a decrease in ET accompanying increasing levels of stimulation. Moreover, we see that at stimulation levels of 8 V/cm, there was some observed lack of contraction, and at 10 V/cm and above, no contractions at all of the engineered cardiac constructs. Maximum capture rates (Fig. 4B) also increased as compared to control for stimulation levels of 1 V/cm but at stimulation levels greater than this decrease (5 V/cm), and then begin to fail (8 V/cm), and finally again, not contract at all (10 V/cm, 12.5 V/cm).

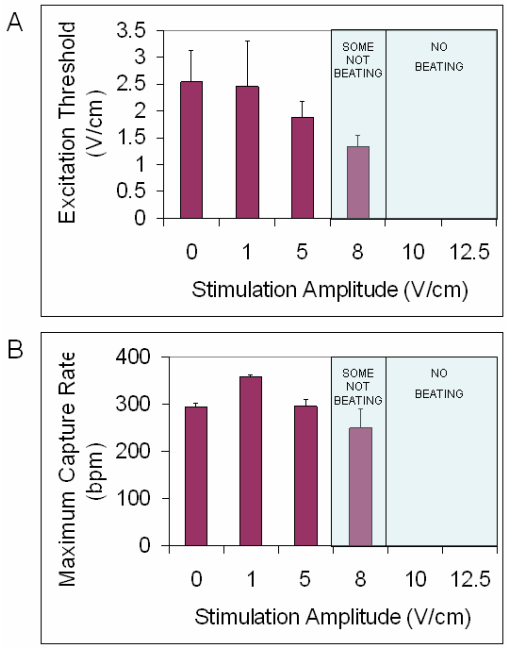

Fig. 4: (A) The measured excitation thresholds and (B) maximum capture rates for scaffolds stimulated at various levels in culture. There is a decrease in excitation threshold accompanying increasing levels of electrical stimulation, although at stimulation levels above 8 V/cm, there was some observed lack of contraction, suggesting that there is an optimal level of stimulation below this level. Maximum capture rates also increased for stimulation levels less than 5 V/cm, but decreased for stimulation at 8 V/cm, and then at levels above this scaffolds would not contract, again suggesting that optimal stimulation levels are below 8 V/cm (n = 3-5 samples per condition).

III. DISCUSSION

There are several important conclusions we can draw from the electrode configuration studies shown in Fig. 3A. The first is that not all electrode configurations are created equal. The increasing R^2 value from the data fitted to a line for 1 cm electrodes as compared to that for 4 cm and 8 cm electrode lengths points to the increasing validity of the approximation of the electrode configurations as parallel plates. In addition, the values for the ratios of the slopes of the lines corresponding to 4 cm and 8 cm electrodes to that of their theoretical lines are close enough (3.2 and 2.8, respectively) that perhaps this difference can be explained by human error in cutting or placing the electrodes, and if so, may indicate that an empirical factor of approximately 3 can adjust for discrepancies between these types of configurations and the assumptions of the parallel-plate model we used (electrode area underestimated because of surface roughness, for example). Secondly, it seems that there are ranges of configurations that seem to be relatively equivalent to each other, as long as the ratio of length to electrode spacing is sufficiently large (approximately greater than 2, corresponding to 4-cm-long electrodes with 2-cm spacing). And so, because it is a good candidate, and also compatible with our cell studies (due to size of scaffolds and

Petri dish), for all further assessments, we chose the configuration of 4-cm-long electrodes with 1 cm spacing (in 60-mm-diameter Petri dishes).

Both electrical measurements and biological data indicate that electrical stimulation of cells during culture with carbon electrodes is safe for cardiac cells. Nevertheless, we are aware that the only truly safe configuration in terms of electrical stimulation is that which shields cells through the use of salt bridges [9]. However, the benefits of a salt-bridged system must be weighed against the drawbacks of added complications and therefore higher risk for disruption of electrical stimulation or even contamination.

A. Recommendations

In this study, we see that electrode configuration strongly affects stimulation efficiency: in particular, that the longer the electrodes are in relation to their spacing, the more "ideal" the configuration. For this reason, we recommend that electrode length be at least twice that of electrode spacing. One possible explanation for the increased deviation of the 1 cm electrodes from theoretical values is that electrode measurements for this length electrode were performed in a 60 mm Petri dish, and there may have been significant edge effects contributing to the measured electrical circuit parameters. In order to prevent this type of problem from interfering with electrical stimulation of cells during culture, we recommend having electrode length be as close to that of the diameter of the Petri dish as possible, placing electrodes on the bottom surface of the Petri dish as well as covering the electrodes with medium, but not putting much more, all to aid in constraining field lines to between electrodes.

As previously shown, after a single experiment, the R_p of the electrodes studied here do not change [7]. However, it is important to note that both η and R_s decrease, indicating that some surface area may be eroded during an experiment, and that the CPE increases. All these points suggest that electrodes should be changed between each experiment, in order to maximize efficiency of the electrical stimulation. In addition, using fresh electrodes for each experiment will decrease risk for contamination.

In this study we have also identified a safe stimulation range using carbon rod electrodes, and at this point we do not recommend stimulation at levels of 8 V/cm and above, although we may refine this range in the future.

B. Future Work

Refining this range of recommended ratio of electrode length-to-spacing will only be possible with more data points characterizing intermediate geometries. Because of the time-consuming nature of EIS measurements over low frequencies, these measurements would be facilitated by either narrowing the frequency range over which EIS measurements are obtained (especially by eliminating measurements at the lowest frequencies) or eliminating the number of intermediate points. Future work also involves construction of stimulation chambers with better-defined geometries. In addition, although in this study we have identified a safe stimulation range, ongoing work includes further optimization of stimulus amplitude as well as frequency (manuscript in preparation).

C. Conclusions

For applications involving bioreactors and/or the application of physical forces, such as the studies outlined here, we must expand our concept of biocompatibility to encompass not only benign passive host response but to also include efficient charge injection with tolerable amounts of reaction products. In addition, electrodes must exhibit sufficient mechanical properties to be included in a bioreactor, and be arranged in a configuration appropriate for the cells being stimulated. Given the highly non-linear behavior of electrochemical systems such as the ones described here, we hope that studies such as these will aid others in bioreactor and experimental setup design as they have helped us.

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