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Characterization of Electrical Stimulation Electrodes 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. The goal of this study was to assess the conditions of electrical stimulation with respect to the electrode geometry, material properties and charge-transfer characteristics at the electrode-electrolyte interface. We compared various biocompatible materials, including nanoporous carbon, stainless steel, titanium and titanium nitride, for use in cardiac tissue engineering bioreactors. The faradaic and non-faradaic charge transfer mechanisms were assessed by electrochemical impedance spectroscopy (EIS), studying current injection characteristics, and examining surface properties of electrodes with scanning electron microscopy. Carbon electrodes were found to have the best current injection characteristics. However, these electrodes require careful handling because of their limited mechanical strength. The efficacy of various electrodes for use in 2-D and 3-D cardiac tissue engineering systems with neonatal rat cardiomyocytes is being determined by assessing cell viability, amplitude of contractions, excitation thresholds, maximum capture rate, and tissue morphology.**

I. INTRODUCTION

NATIVE heart tissue has low resistance for electrical signal propagation due to presence of gap junctions and high cell density. Individual cells are packed together at a density on the order of 100 million cells per cm3 tissue volume, and held in place by tight junctions, such that the myocardium acts as a synctium (1-3). In native heart, mechanical stretch is induced by electrical signals, and the orderly coupling between electrical pacing signals and macroscopic contractions is crucial for the development and function of native myocardium.

To engineer functional cardiac constructs, cell populations isolated from neonatal rat heart ventricles are cultured on a biomaterial scaffold (providing a highly porous, elastic, biodegradable template for tissue formation) in a bioreactor (providing environmental control and the application of molecular and physical regulatory signals). To enhance functional cell assembly, we induced synchronous contractions of cultured cardiac constructs by applying electrical signals designed to mimic those orchestrating the synchronous contractions of cells in native heart (4).

Over only 8 days *in vitro*, electrical field stimulation resulted in cell alignment and coupling, markedly increased the amplitude of synchronous construct contractions and resulted in a remarkable level of ultrastructural organization (4).

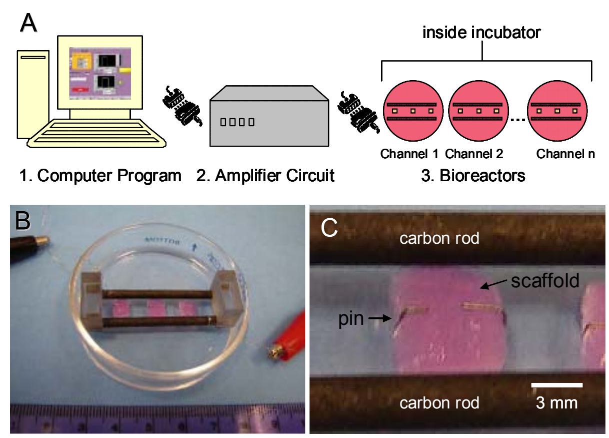

Fig. 1 Experimental setup for supra-threshold stimulation of cardiac myocytes. A. Electrical stimulation voltages are set using a computer program, output through 8-channel AO card, amplified, and interfaced to bioreactors. B. 60 mm Petri dish with carbon rod electrodes, spaced 1 cm apart. C. Close up view of scaffold positioned between electrodes and held in place with two stainless steel pins.

Electrical stimulation also promoted cell differentiation and coupling, as evidenced by the presence of striations and gap junctions, and resulted in concurrent development of conductive and contractile properties of cardiac constructs (4).

The present study was focused on the conditions of electrical stimulation, and aimed at developing rational design principles for the selection of stimulation electrodes.

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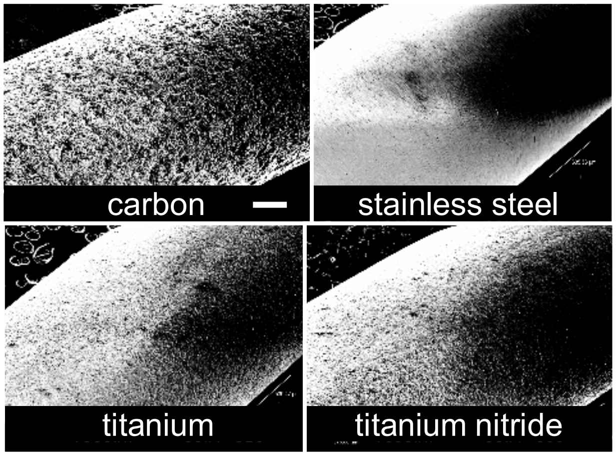

Fig. 2 Scanning electron microscopy (SEM) images of electrodes made of the four materials tested: nanoporous carbon, stainless steel, titanium, and titanium nitride. Each electrode is in a form of a 3 mm diameter rod. Scale bar corresponds to 500 µm.

II. MODEL SYSTEM

Our overall approach to electrical stimulation is biomimetic in nature, as it is designed to recapitulate some aspects of the actual in vivo environment:

- 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), and supplementation of oxygen carriers (to mimic the role of hemoglobin)
- Induction of macroscopic synchronous contractions of cultured constructs by electrical signals designed to mimic those in native heart.

We focus here on the electrical stimulation component of our model system, designed to promote orderly coupling between electrical signals and cell contractions in a way similar to that in native myocardium (5).

Cardiac constructs were prepared by seeding collagen sponges (6 mm x 8 mm x 1.5 mm) with cell populations isolated from neonatal rat heart ventricles (5 million cells per scaffold, corresponding to the initial density of 80 million cells per cm3 total volume) and stimulated using supra-threshold square biphasic pulses (2 ms duration, 1 Hz, 5 V).

During bioreactor cultivation, tissue constructs are maintained in a constant position, aligned with the direction of the electrical field gradient. Constructs contract synchronously in response to electrical field stimulation, and these contractions should not be constrained. To meet both requirements, constructs are kept aligned using stainless-steel pins, held in place by a thin layer of PDMS (Fig 1).

III. SELECTION AND CHARACTERIZATION OF ELECTRODES

The design considerations required for electrical stimulation of cardiac tissue constructs include the duration and shape of the stimulus waveform, the size of the tissue construct, the oxygen species or firing of the action potential, the duration of cultivation, and the mechanical properties required of the electrode to function within a given bioreactor setup (6).

Our first objective was to assess the conditions of electrical stimulation for several candidate materials that can be used to fabricate electrodes within cardiac tissue engineering bioreactors. We compared four biocompatible materials: nanoporous carbon, stainless steel, titanium, and titanium nitride with respect to the material properties and charge-transfer characteristics at the electrode-electrolyte interface. The electron micrographs of the electrodes made of the four different materials that were tested are shown in Fig. 2.

Electrical stimulation needs to be applied such that a desired physiological response can be attained with none or only minimal damage to the cells. For each application, one needs to choose appropriate electrode material, geometry and charge-transfer characteristics at the electrode-electrolyte interface. Electrodes must be biocompatible to avoid toxic or immune responses in the adjacent tissue or medium, and they should efficiently transfer charge from the electrode material where it is carried by free electrons to the medium or tissue where it is carried by ions.

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. The first two mechanisms are desirable, while the last should be avoided because it is associated with electrode degradation and harmful byproducts. The relative presence of each mechanism can be assessed using electrochemical impedance spectroscopy (EIS), from which an equivalent circuit of the stimulation system can be constructed (6).

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. Equivalent circuits and associated parameters are determined using ZView 2.5b as previously described (7-9).

EIS spectra were acquired for each electrode in a Petri dish containing 20 ml of PBS, over a frequency range from 1 x 106 to 1 x 10–2 Hz, with a perturbation amplitude of 10 mV. For each frequency, the real (resistive) component of the impedance response (Z') and the imaginary (capacitive) component of the impedance response (Z") were recorded. Collected data were evaluated in ZView to generate Nyquist plots for each condition. Fig. 3 shows the Z' – Z" Nyquist plot for the four electrodes studied. The shape of the curves is indicative of the presence of reactions (high for stainless steel, low for other electrode types) (6).

Fig. 4 shows an "equivalent circuit" of the system created using resistors Rp (polarization resistance) and Re (electrolyte resistance) and a capacitor-like "constant phase element" (CPE) in series and in parallel.

Fig. 5 shows a Bode plot that gives the logarithm of impedance and the phase angle as a function of frequency.

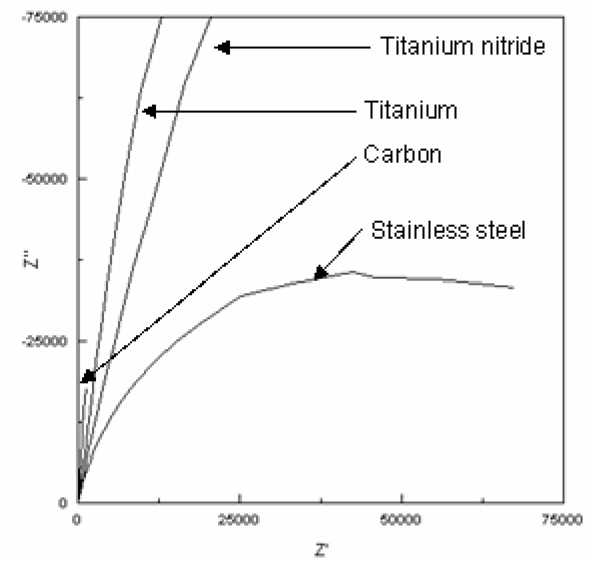

Fig. 3 Nyquist plots of the four electrode types. The semicircular shape of the Nyquist plot for stainless steel suggests the presence of reactions. In contrast, titanium, titanium nitride, and carbon electrodes have linear profiles associated with high polarization resistance.

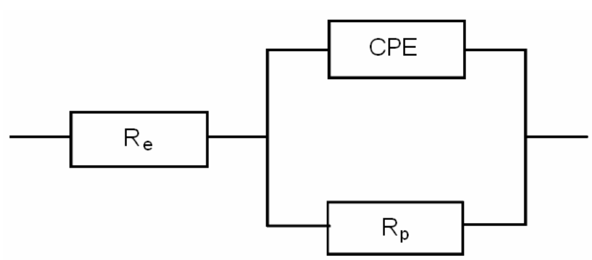

Fig. 4 Equivalent circuit model of the electrode/electrolyte interface. Rp is the polarization resistance, CPE is a constant phase element, and Re is electrolyte resistance

The values shown in Table 1 for CPE, Rp and η (a term indicating the non-ideality of CPE, ranging from 0 to 1 for an an ideal capacitor) were then calculated using instant fit functions in ZView software. The relatively low value of Rp for stainless steel electrode confirms that this electrode is more susceptible to faradaic reactions, and hence corrosion. In contrast, the carbon electrodes are well suited for electrical stimulation, because their very high Rp value minimizes faradaic reactions, and their relatively high CPE value allows high charge transfer to the electrolyte (culture medium), and hence to the tissue construct.

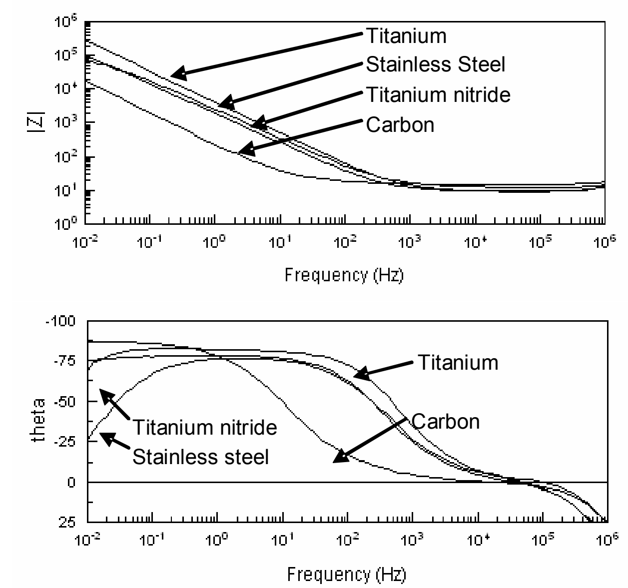

Fig. 5 Bode plots. Carbon electrodes have the lowest impedance modulus |Z| across all frequencies. Experimental setup was as shown in Fig. 1 with 4 cm electrodes and 10 mV perturbation.

Material	**CPE, /cm^2** (% error)	**R_p, Ω/cm^2** (% error)	**η** (% error)
Carbon	1.90E-4 (1.27%)	4.06E+13 (0.05%)	0.91 (0.46%)
Stainless Steel	2.30E-5 (1.55%)	2.17E+4 (4.76%)	0.85 (0.41%)
Titanium nitride	2.66E-5 (0.36%)	4.68E+5 (21.9%)	0.86 (0.10%)
Titanium	1.12E-5 (0.49%)	4.09E+5 (9.91%)	0.91 (0.11%)

Table 1: Calculated values of the CPE, Rp and η for the four electrodes. The relatively low value of Rp for stainless steel confirms that it is more susceptible to faradaic reactions, and hence corrosion. At the other extreme, the carbon electrodes are best suited for electrical stimulation: their very high Rp value minimizes faradaic reactions and the relatively high CPE value indicates that the electrode transfers more charge to electrolyte, and hence tissue construct. The η term indicates non-ideality of CPE, and ranges from 0 to 1 for an an ideal capacitor.

In summary, electrical stimulation was shown to markedly enhance functional assembly of isolated heart cell populations cultured on a 3D scaffold. These findings motivated in-depth studies of the conditions of electrical stimulation, with respect to the electrode geometry, material and charge-transfer characteristics at the electrode-electrolyte interface. In the present work, we compared electrodes made of nanoporous carbon, stainless steel, titanium and titanium nitride. The faradaic and non-faradaic charge transfer mechanisms were assessed by electrochemical impedance spectroscopy (EIS). Carbon electrodes were found to have the best charge transfer.

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