

Testing electrode suitability for field stimulation of high-threshold biological preparations

Hugo Fernando Maia Milan*, Rosana Almada Bassani, José Wilson Magalhães Bassani

Abstract Introduction: A problem posed by electrical field (E) stimulation of biological preparations with high excitation threshold is that the E intensity required for excitation is likely to induce water electrolysis at the electrode surface, which can alter the extracellular medium and cause deleterious effects on the cells. In this study, different electrode materials and geometries were tested aiming at identifying electrode configurations that could transduce the E intensity required for exciting ventricular cardiomyocytes isolated from neonatal rats (threshold $E \sim 30$ V/cm) without causing water electrolysis. **Methods:** Wire and plate electrodes made of platinum, stainless steel and nickel/chrome alloy were used. The effect of blasting the electrode surface with sand and NaHCO_3 solution was also tested. Electrodes were inserted into a cell perfusion chamber containing the saline solution routinely used for physiological experiments. During E application for 5 min, the electrode surface and its surroundings were examined at high magnification for the presence of microbubbles, which indicates the occurrence of water electrolysis. The greatest E intensity applied that failed to generate microbubbles (E_n) was estimated. **Results:** While nickel/chrome and stainless steel electrodes resulted in low E_n values, the best performance was observed for sandblasted platinum wire (2 mm diameter) and plate (25 mm x 5 mm; 0.1 mm thickness) electrodes, for which E_n was ≥ 40 V/cm. **Conclusion:** These electrode configurations are suitable for effective and safe stimulation of isolated neonatal cardiomyocytes.

Keywords: Electrodes, Field stimulation, Isolated neonatal cardiomyocyte, Water electrolysis.

Introduction

Stimulation with external electrical fields (E) is a common experimental procedure to trigger action potentials in excitable preparations, or to evoke neurotransmitter release from terminals that innervate biological tissues (e.g., Fonseca et al., 2013; Gomes et al., 2002; Merrill, 2011; Zafalon et al., 2013). High intensity E stimulation may also be used to induce electroporation (Goulart et al., 2012; Maswiwat et al., 2008; Oliveira et al., 2008). However, using high E intensities may represent a problem, not only regarding the limited availability of electrical stimulators that can supply the necessary stimulus amplitude, but mainly because of undesirable effects related to the flow of high intensity electrical currents across the stimulating electrodes immersed in an aqueous, saline medium.

While electrons are the main charge transporters in electrical circuits, in saline solutions that do not contain free electrons, charge is transported predominantly by ions (Cogan, 2008). If the intensity of the charge transduced from electrical current (in electrical circuits) to ionic current (in saline solutions) is below a threshold value, called safe value (Merrill, 2011), chemical processes occurring during charge flow are

reversible. This means that the chemical reactions that take place during the positive phase of a symmetrical biphasic pulse will be reverted during the negative phase (Brummer and Turner, 1977). However, if the transferred charge is above the safe value, irreversible electrochemical reactions, such as water electrolysis, may occur. During water electrolysis, O_2 gas is produced at the anode, whereas H_2 gas is produced at the cathode, as a result of oxidation and reduction, respectively, whereas the local pH is decreased at the anode and increased at the cathode (Donaldson and Donaldson, 1986). The changes in pH and formation of reactive free radicals, as well as diffusion through the solution of the generated gases contained in the bubbles, may affect the function of the biological preparation under study, and cause deleterious effects (Merrill, 2011).

As the safe value of charge transduction depends not only on the electrode material, but also on its shape (Feltham and Spiro, 1971), the aim of this study was to test different electrode materials and geometries, in the search for the most suitable combination that allows greater stimulus intensity and reduced probability of water electrolysis. We took as a reference the E

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intensity required for suprathreshold stimulation of ventricular cardiomyocytes isolated from neonatal rats, which is much greater than that in adult cells (30 vs. 3-6 V/cm, Gomes et al., 2001; Goulart et al., 2012).

Methods

Electrodes were tested in a cell perfusion chamber reported elsewhere (Gomes et al., 2001; Oliveira et al., 2008). The chamber perfusion/stimulation area was oblong, with 25 mm length, 7.5 mm width and 4 mm depth. The electrodes were inserted along the side walls of the chamber, parallel to the chamber major axis (Figure 1).

The tested materials for electrode manufacturing were nickel/chrome alloy (0.6 mm diameter wires), stainless steel (0.5 or 1 mm diameter wires), and platinum. The latter was used as a wire (diameter of 0.25, 0.5 or 2 mm), or a 0.1 mm thick plate measuring 5 mm x 25 mm. The length of all wire electrodes was the same as that of the perfusion chamber (25 mm). Some platinum electrodes were blasted with sand or NaHCO_3 solution to increase their surface area (Brummer and Turner, 1977).

The chamber was filled with 0.5 ml of Tyrode's solution (millimolar composition: 140 NaCl; 6 KCl; 1.5 MgCl_2 ; 5 HEPES; 1 CaCl_2 ; 11 glucose; pH 7.4) with electrical conductivity of 1.4 S/m (Gomes et al., 2001). Biphasic symmetrical, square voltage pulses (10 ms total duration, 0.5 Hz) generated by an electrical stimulator developed at the Center

for Biomedical Engineering of the University of Campinas (CEB/UNICAMP) were applied to the electrodes. The electrical potential in the chamber was measured with Ag/AgCl electrodes connected to a digital oscilloscope (mod. TDS-360, Tektronix Inc., Beaverton, OR, USA). These electrodes were positioned at different points along the chamber midline, perpendicularly to the electrode length (Figure 1). The E intensity was calculated as the ratio of the potential difference measured at two points and the distance between them, and the estimates for different measuring points were averaged. As seen in previous study (Oliveira et al., 2008), E was considerably uniform, varying less than 2% near the chamber center. After 5 min stimulation, the surface of the electrodes and the surrounding regions were examined using a microscopy system (Ricardo et al., 2006) under 285X (or 1060X, if necessary for confirmation) magnification, in the search for microbubbles, which were considered an indicator of water electrolysis (Donaldson and Donaldson, 1986). Initially, 1 V pulses were applied to the electrodes. If microbubbles were not observed, the procedure was repeated increasing the stimulus amplitude in 1 V steps until they were detected. Then, the test was interrupted and the E value calculated in the previous stimulation trial was considered as the greatest E intensity not able to cause water electrolysis (E_n). A single experiment was performed for each electrode configuration.

Results

Table 1 shows the calculated E_n values for the different tested electrodes. For the same wire diameter, E_n was 2-3 times greater for platinum electrodes than for electrodes made of other materials. For the wires, it was observed that the greater the diameter, the larger was E_n . Blasting platinum electrodes resulted in an increase in E_n , which was twice as great for sand as for NaHCO_3 blasting. Among the tested combinations, the greatest E_n values were observed using sandblasted platinum plates and 2 mm diameter wires ($E_n > 30$ V/cm).

Discussion

Among the tested materials, platinum electrodes showed the best performance, with the highest E_n values, and more so for larger, blasted electrodes, probably due to increase in the electrode surface area. The superiority of platinum was not surprising because it is one of the pure metals with greatest capacity for transducing charge without development of irreversible chemical reactions (Cogan, 2008)

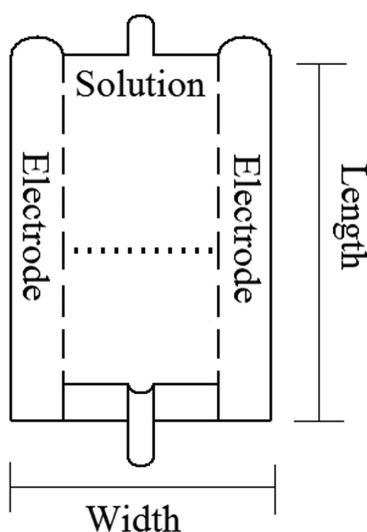


Figure 1. Schematic model of the perfusion chamber. The stimulation electrodes were placed along the chamber side walls, touching the chamber bottom. The dotted line at the middle of the chamber indicates where the electrical potential was measured for estimation of the field intensity.

Table 1. Maximum value of external electrical field at which microbubbles (water electrolysis indicator) were not formed (E_n) for stimulation electrodes of different materials and configurations.

Electrode geometry	Polish	Material	Diameter (mm)	E_n (V/cm)
Wire	None	Nickel/chrome alloy	0.6	2.5
Wire	None	Stainless steel	0.5	3.5
Wire	None	Stainless steel	1.0	10.1
Wire	None	Platinum	0.25	2.6
Wire	None	Platinum	0.5	7.6
Wire	NaHCO ₃	Platinum	0.5	11.2
Wire	Sandblast	Platinum	0.5	17.2
Wire	None	Platinum	2	21.6
Wire	NaHCO ₃	Platinum	2	31.0
Wire	Sandblast	Platinum	2	40.8
Plate	Sandblast	Platinum	*	43.6

*5 mm height, 0.1 mm thickness, 25 mm length.

due to its high capacity of adsorbing hydrogen ions (hydrogen atom plating; Brummer and Turner, 1977).

Considering that the threshold E value for excitation of neonatal rat cardiomyocyte is ~ 30 V/cm (Gomes et al., 2001), our results show that, among the tested electrodes, only the sandblasted platinum plate and the wire with the greatest diameter (2 mm) resulted in E_n values compatible with safe suprathreshold stimulation (≥ 40 V/cm), *i.e.*, without production of detectable water electrolysis. While the wire is more robust for daily use than the plate, it should be noticed that the metal volume required for its confection is ~ 76 -fold greater, which implies in greater cost. On the other hand, the use of stainless steel and nickel-chrome alloy wires with less than 1 mm diameter is not advisable for E stimulation even of adult cardiomyocytes, which present a relatively low threshold (3-6 V/cm; Gomes et al., 2001; Goulart et al., 2012).

One of the limitations of this study is that a single test was performed with each electrode configuration, which prevented statistical comparison. However, the differences among the E_n values were sufficiently large to allow easy distinction of the best electrode types for the intended use.

In conclusion, in the present study it was possible to identify electrode configurations that allow the application of high-intensity, stimulating electrical fields without production of significant water electrolysis. Our results showed that suprathreshold stimulation can be safely applied to neonatal cardiomyocytes ($E > 30$ V/cm) through sandblasted platinum plate or wire electrodes with at least 2 mm diameter. If greater E values are required for stimulation, one may attempt increasing further the electrode surface area and/or resorting to advanced electrode manufacturing and coating techniques (Merrill, 2011).

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