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Low level direct current — cell culture fibroblast model

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Abstract

Influence of low level direct current (DC) on cell growth was studied on different cell culture models. V-79-379 A cells were treated with direct current (0.2–1.5 mA) for 1 h in cell suspension or in cell monolayer. DC was delivered through Pt–Ir electrodes: (i) in direct contact to the cell suspension; (ii) in direct contact with culture medium and cell monolayer in Demetri dish and (iii) indirectly in a Petri dish where electrodes were separated from the cell monolayer by two agar bridges. The effects of direct current immediately and 48 h after treatment on cell growth were evaluated by cell counting. The results indicate that the cytotoxic effect of DC was independent on whether the cells were treated in the suspension or in cell monolayer. Strong inhibitory effect on cell growth was diminished when DC was applied through agar bridges.

Keywords: Direct current; V-79 cells; Cell growth

1. Introduction

Electrical currents may enhance healing of chronic wounds [1] and retard tumor growth [2]. Our previous results indicate that low level direct current (DC) has an effect on the cell growth of normal (V-79) [3] and tumor cell lines (SA-1 and B16) [4]. DC probably acts directly on the cell growth and/or indirectly through cell growth media. It was also reported by Rosenberg et al. [5] and Mortensen and Bojsen [6] that electrolysis products from a platinum electrode can cause the inhibition of cell division in *Escherichia coli* and human bone marrow cells respectively. To test these findings three different models were designed: two models with electrodes in direct contact to the culture medium (cell suspension or cell monolayer) and one where electrodes were separated from the cell monolayer by two agar bridges.

2. Material and methods

2.1. Cells

The cells V-79-379 A (diploid lung fibroblasts of Chinese hamster) were grown in Eagle MEM, supplemented with 10% fetal calf serum (FCS) penicillin (100 U ml^{-1}) and streptomycin (100 μg ml^{-1}) at 37°C in a CO_2 incubator.

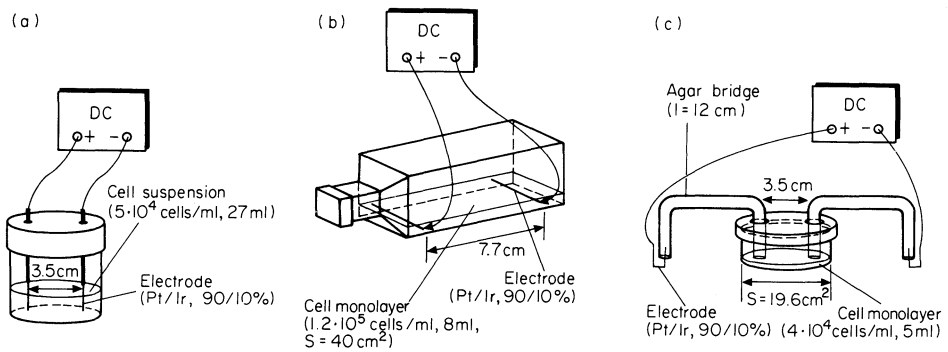

Fig. 1. (a) Electrodes in direct contact to the cell suspension in the culture medium. (b) Electrodes in direct contact to the culture medium and cell monolayer. (c) Electrodes were separated from the cell monolayer by agar bridges.

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2.2. Direct current treatment

DC (0.2–1.5 mA) was delivered through Pt–Ir (90–100%) alloy electrodes for 1 h. Three different cell culture models were established.

Model 1: Electrodes in direct contact to the cell suspension in culture medium (Fig. 1(a)). The cells were suspended in culture medium without FCS (27 ml, 5×10^4 cells ml^{-1}) and placed in glass dishes (radius 21 mm, height 40 mm) with a teflon cover in which electrodes were fixed (the distance between electrodes was 35 mm). During the treatment (0.2–1.5 mA, 3.2–3.8 V for 1 h) the dishes were placed on a shaker to prevent cell adhesion. Immediately after treatment with DC the cells were detached with 0.25% trypsin solution and counted in a hemocytometer and the cell number: control (%) ratio calculated. Thereafter the cells were transferred to the tissue culture dishes in equal numbers for determination of cell growth. For this purpose cells were counted in a hemocytometer after 48 h and the cell number: control (%) ratio was again calculated.

Model 2: Electrodes in direct contact to the culture medium and cell monolayer (Fig. 1(b)). Cells were grown in cell monolayer (1.2×10^5 cells ml^{-1}) in Demetri dish (8 ml, 40 cm^2) with electrodes immersed into the culture medium in a Demetri dish. After the treatment (0.2–1.5 mA, 3.1–3.7 V for 1 h) the cells were detached with 0.25% trypsin solution and counted in a hemocytometer and cell number: control (%) ratio was calculated.

Model 3: Electrodes were separated from the cell monolayer by agar bridges (Fig. 1(c)). Cells were grown in a Petri dish (19.6 cm^2 with 5 ml culture medium) in cell monolayer (4×10^4 cells ml^{-1}) and treated with DC through electrodes, separated from the cell monolayer by two 12 cm long agar bridges. The effect of DC (0.2–1.5 mA, 5.7–20 V for 1 h) immediately after treatment and cell growth after 48 h were measured by cell counting. Cells were detached with 0.25% trypsin solution and counted in a hemocytometer and cell number: control (%) ratio was calculated.

In control experiments the cells were treated in the same way as in experimental samples with electrodes introduced into the cell culture medium or through agar bridges for the same period of time (1 h), except that no current was delivered.

3. Results

The effect of direct current immediately after treatment on V-79 cells, treated with electrodes in direct contact with cell suspension and cell monolayer (Models 1 and 2) is shown in Fig. 2. Immediately after treatment with DC, the cells were counted in a hemocytometer. The cytotoxic effect on the cells immediately after treatment was observed regardless of whether the cells were treated in suspension or cell monolayer and was dose dependent. The cell number decreased to 60% of the control value at 1 mA and 45% at 1.5 mA.

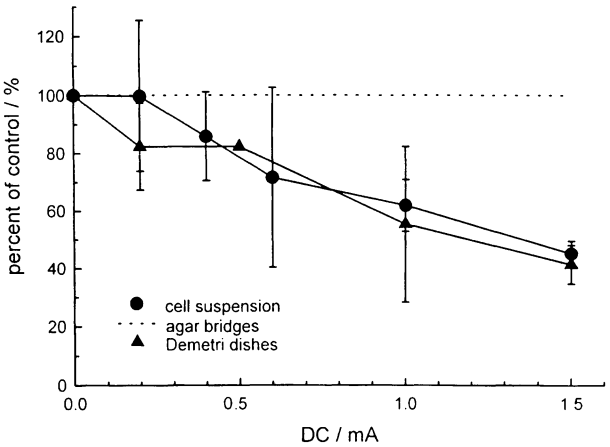

Fig. 2. The effect of DC immediately after treatment.

When the electrodes were separated from the cells by agar bridges (Model 3) cells remained attached and cell number unchanged.

Figure 3 represents the effect of DC on cell proliferation. Cells treated in cell suspension (Model 1) and with electrodes separated from the cell monolayer by agar bridges (Model 3) were grown for 48 h and net cell increase was determined by cell counting and the cell number: control (%) ratio was calculated.

The inhibitory effect of DC treatment on cell growth after 48 h of the cells treated in cell suspension was dose dependent (25% at 1.5 mA) and more pronounced in comparison to agar bridges.

Strong inhibitory effect on cell growth was diminished when DC was applied through agar bridges (Model 3). Weak inhibitory effect was observed at 1 mA treatment but results were very dispersed.

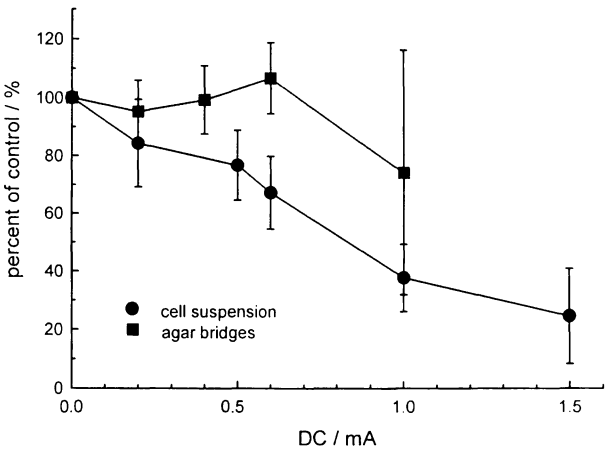

Fig. 3. The effect of DC on cell proliferation.

4. Discussion

Three different models were designed to test the direct (electrodes immersed into the culture medium) and indirect (agar bridges) effects of low level direct current on cell suspension and cell monolayer. Immediate effect was expressed only when the electrodes were in direct contact to the culture medium regardless of whether the cells were in suspension or monolayer. At 1.5 mA only 45% of control value was detected after treatment. Cell number remained unchanged when the electrodes were separated from the cells by agar bridges. When 48 h growth was compared between models 1 and 3, a strong inhibitory effect was detected when the cells were treated directly in the cell suspension in comparison with agar bridges. In the former model cell number decreased to 63% of control value at 0.6 mA and 38% at 1 mA treatment while in the latter the cell number remained unchanged at currents 0.6 mA and lower and decreased to 75% at 1 mA treatment. For each model the possible temperature rise due to current flow was estimated. Assuming that the cell culture media were ideally thermally insulated from the surroundings and that all of the electric energy delivered was converted to heat, the temperature rise would be:

Model 1 temperature rise (worst case):

$\Delta T = 20.52$ Watt sec (27g, 4.175 Watt sec g^{-1} °K)$^{-1}$ = 0.18°K,

Model 2 temperature rise (worst case):

$\Delta T = 19.98$ Watt sec (8g, 4.175 Watt sec g^{-1}, °K)$^{-1}$ = 0.6°K,

Model 3 temperature rise (worst case):

$\Delta T = 108$ Watt sec (15g, 5.175 Watt sec g^{-1} °K)$^{-1}$ = 1.7°K, which is probably too low to explain results observed.

Several authors studied the effect of toxic substances released from the electrodes at low level DC treatment which might interfere with biological systems tested (inhibition of growth) [5,6]. Mortensen and Bojsen [6] reported the inhibitory effect of platinum electrodes on human bone marrow cells but no inhibition of growth was found when titanium electrodes were used. The inhibition of cell division in *Escherichia coli* by electrolysis products was detected by Rosenberg et al. [5]. Our previous results [3] were in agreement with results mentioned above showing the effects of DC treatment on cells in suspension. The inhibitory effect on cell growth and cell survival were observed. A slight inhibitory effect of treated culture medium on untreated cells was noticed as well [3]. According to our previous [3] results we proposed that direct current could act on cell growth as well as indirectly through cell growth media via toxic compounds released into the media by electrolysis during DC treatment.

Our results indicate that the effect of DC was independent on whether the cells were treated in the suspension or in cell monolayer. Cell adherence to the culture dish and cell growth did not affect DC influences. Our results show that the observed cytotoxic effect of DC was due to electrochemical reaction products at the electrode–electrolyte interface (Fig. 2). The proposed inhibitory effect of DC on cell growth was, though noticable, only partially confirmed (Fig. 3).

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