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Bioelectrochemistry 70 (2007) 519 – 523
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Short communication

Increase of the roughness of the stainless-steel anode surface due to the exposure to high-voltage electric pulses as revealed by atomic force microscopy

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Received 24 March 2006; received in revised form 4 December 2006; accepted 15 December 2006

Abstract

The changes of the stainless-steel electrode surface morphology occurring due to dissolution of the anode under the action of electric pulses which are commonly utilized in cell electromanipulation procedures, have been studied by using atomic force microscopy. The surface of the polished electrode was rather smooth — the average roughness was 13–17 nm and the total roughness 140–180 nm. After the treatment of the chamber filled with 154 mM NaCl solution to a series of short (about 20 μs), high-voltage (4 kV) pulses, the roughness of the surface of the anode has increased, depending on the total amount of the electric charge that has passed through the unit area of the electrode, and exceeded 400 nm for the dissolution charge of 0.24 A s/cm^2. No changes of the cathode surface were detected. Well-defined peaks with the width of 1–2 μm and the height of over 400 nm have appeared. These peaks create local enhancements of the electric field at the interface between the solution and the electrode surface which can lead to the non-homogeneity treatment of cells by electric pulses and can facilitate the occurrence of the electrical breakdown of the liquid samples.

Keywords: Surface topography; Electrolysis; Electroporation; Arcing; PEF food processing

1. Introduction

A temporary increase of cell membrane conductivity and permeability (electroporation) is achieved by the exposure of cells suspended in an electrolyte solution to pulses of strong electric field (up to 100 kV/cm) [1]. This phenomenon is widely used for cell electromanipulations in biomedicine, cell biology, biotechnology, and food processing [2,3].

However, when a high enough voltage is applied to the electrolyte solution, besides a permeabilization of the cell membrane, a variety of electrolysis reactions occur at the electrode–solution interfaces [4]. These reactions cause changes of the various parameters of the experimental medium, such as the chemical composition [5–7] or pH [8]. When a non-inert anode is utilized, the dissolution of the anode material might occur due to the oxidation of the metal of the electrode at the anode–solution interface [4].

Studying the processes of electrolysis occurring during cell electroporation procedures became especially important when electroporation was recently started to be used *in vivo* for electrochemotherapy [9], transdermal drug delivery [10], gene therapy [11], as well as nonthermal pasteurization of liquid foods [3,12] as the interaction between electrode materials and tissues or food products during electric treatment should be minimized [3].

One of the problems that sometimes arise when using the pulses of as high electric field as possible for food pasteurization or other applications, is so called “arcing”, that is, the dielectric breakdown of the liquid samples which is observed as a spark [13–16]. This can lead to dramatic effects from just ejection of the sample from the cuvette [14] or the reduction of the transfection efficiency [17] to irreversible damage of electroporation cuvettes, power supplies or electroporators [16,18]. Reducing the risk of dielectric breakdown of foods during

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doi:10.1016/j.bioelechem.2006.12.003

pulsed-electric-field application is a key issue to success of pulsed-electric-field pasteurization technology [13].

Meanwhile, when using electroporation for the electroporative gene DNA transfer [19], microbial inactivation by pulsed electric fields [12], or other applications, it is often necessary to apply the pulses of as high electric field as possible [20]. In addition, the ultimate goal in some applications, such as electrochemotherapy and food pasteurization by pulsed electric fields, is to kill as many tumor cells or microorganisms as possible [21]. To achieve this, each cell has to be exposed to an electric treatment of sufficient intensity and any inhomogeneity of an electric field is undesirable [21,22]. So, for preventing dielectric breakdown inside the treatment chamber and assuring the homogeneity of an electric treatment of each cell, the variations of the local electric field strength in the treatment chamber must be avoided [21–23]. For example, changing electrode orientation in the case of solid tumors or agitation of the liquid samples allows to achieve more homogeneous electric treatment and improves the efficacy of electrochemotherapy and microorganism inactivation procedures [21,22].

Parallel plates produce the most uniform distribution of electric field [24]. However, long time pulsing and repeatable usage of the electrodes can increase their roughness to such an extent which might create local enhancements of the electric field at the interface between the solution and the electrode surface. This, in some cases, could increase the chance of electrical breakdown of the liquid samples and thus limits the intensity of the electric pulses that could be applied to the treatment chamber [25].

Studying the process of the electrode dissolution is also important in respect to the ability to predict durability of the electrodes utilized in continuous-flow channel chambers [12,20,26,27]. In such systems, electrodes carry a huge load because of repetitive pulsing during the process. To electroporate several tens of millilitres of the cell suspension, it often needs to apply several tens [20] or hundreds [27] of high-voltage electric pulses. Even in the case of the ‘static’ chambers, pulse cycles consisting of up to several thousands of electric pulses are sometimes required to electroporate the cells successfully [28].

Although, there were evidences that cell electromanipulation procedures can result in a substantial release of metal ions from the anode [5–7,29–31] and, in some cases, the cathode [8] and that polishing of the electrodes, which decreases the roughness of their surface, helps to avoid arcing at high voltages [15], no study on the changes of the electrode surface morphology occurring during these procedures has been carried out to date. This study is the first attempt to investigate the changes of the stainless-steel anode surface morphology occurring due to its dissolution under conditions close to those which are used during cell electroporation experiments. The topography of the electrode surface was investigated by means of atomic force microscopy (AFM) — a powerful technique capable of determining the surface topography at a nanometer resolution [32].

2. Experimental

A home-made discharge chamber consisting of two round, flat, parallel electrodes mounted in a well of Teflon was used. The inter-electrode distance was 1 cm and the chamber volume — 2 ml. The only metal parts of the chamber that were in a contact with the solution were the stainless-steel electrodes. The working area (the one through which the electric current was passing) was equal to 2 cm^2.

Prior to the electric treatment, the surfaces of the electrodes were thoroughly polished to get as smooth surface as possible: first, with emery paper (grade 1000 and 1200), then, with diamond paste of decreasing grade (6, 3, 1, and 0.25 μm) till the mirror-like surfaces were obtained. After polishing, the working surfaces of the electrodes were rinsed with alcohol and distilled water.

The dissolution of the electrodes was obtained by discharging several times a high-voltage 1 μF-capacitor (charged to 4 kV) through the chamber filled with a 154 mM NaCl solution. Experiments were done at room temperature (19–20 °C).

The intensity of electrotreatment was characterized by the total amount of electrical charge that has passed through the square unit of the electrode (“dissolution charge”) calculated according to

$$Q_{\mathrm{diss}} = nCU/S$$

Here n is the number of electric pulses, C is the capacity of a capacitor (1 μF), U is the voltage to which the capacitor is charged (4 kV), and S is the electrode area (2 cm^2).

Surfaces of the electrodes were examined in air by atomic force microscope Quesant Qscope-250 (Ambios Technology Company, Santa Cruz, CA, USA). Microfabricated contact mode silicon V-shaped cantilevers with a spring constant of approximately 0.28 N/m were utilized. All images were obtained in a contact mode with the scan rates ranging from 1 to 3 Hz. Repeated scanning of the same place on the electrode surface confirmed that no physical damage had occurred due to measurements.

AFM images were processed and surface roughness parameters were obtained with software SPIP v. 2.3206 (Image Metrology A/S, Lyngby, Denmark). Height profiles were obtained with a Quesant ScanAtomic Control Panel v. 3.20 (Ambios Technology Company, Santa Cruz, CA, USA).

3. Results and discussion

We studied the effect of the short (about 20 μs), high-voltage (4 kV) pulses of high-density electric current (up to $5 \cdot 10^5$ A/m^2), which are commonly utilized in cell electromanipulation procedures, on the surface morphology of the electrodes. Using AFM technology we investigated the morphology of the surface of the electrodes prior to and after the exposure by a series of high-voltage electric pulses.

Despite that the diverse electrode materials are utilized in commercially available and home-made electrodes which are used in cell electromanipulation procedures [33], one of the most popular is still stainless-steel [7,33–35]. Due to this, the electrodes made from stainless steel were chosen as an object of this study.

First, the morphology of the surface of the ‘intact’ electrodes, which were thoroughly polished prior to the electric treatment to get as smooth surface as possible, was investigated. Fig. 1A shows a typical three-dimensional image of the surface of the freshly polished stainless-steel electrode. The scanning area was 30×30 μm^2. The height profile of the electrode surface along the white line is shown in Fig. 2A.

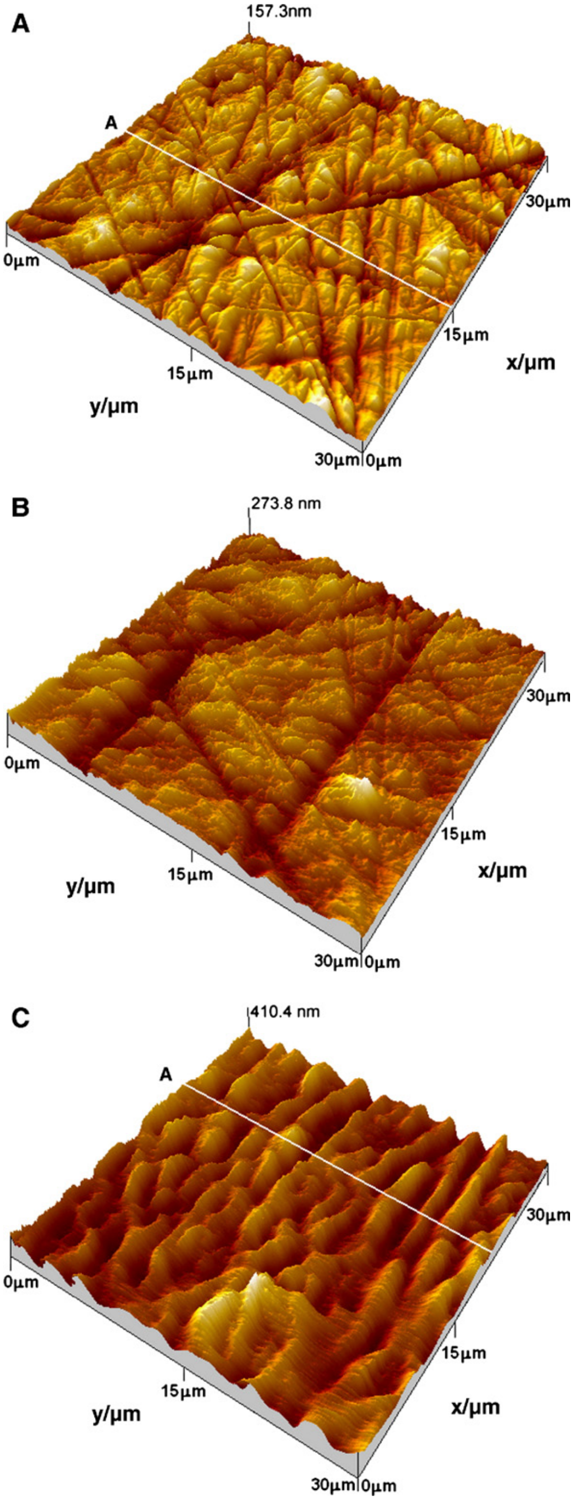

Fig. 1. Typical three-dimensional AFM images of the surface of the stainless-steel anode: (A) polished anode prior to the exposure by high-voltage electric pulses and after the exposure to (B) 60 and (C) 120 exponential pulses with the duration of about 20 µs (dissolution charge $Q_{\mathrm{diss}}=0.12$ and 0.24 A s/cm^2 respectively). Scanning area 30×30 µm^2; z range: (A) 157, (B) 274, and (C) 410 nm.

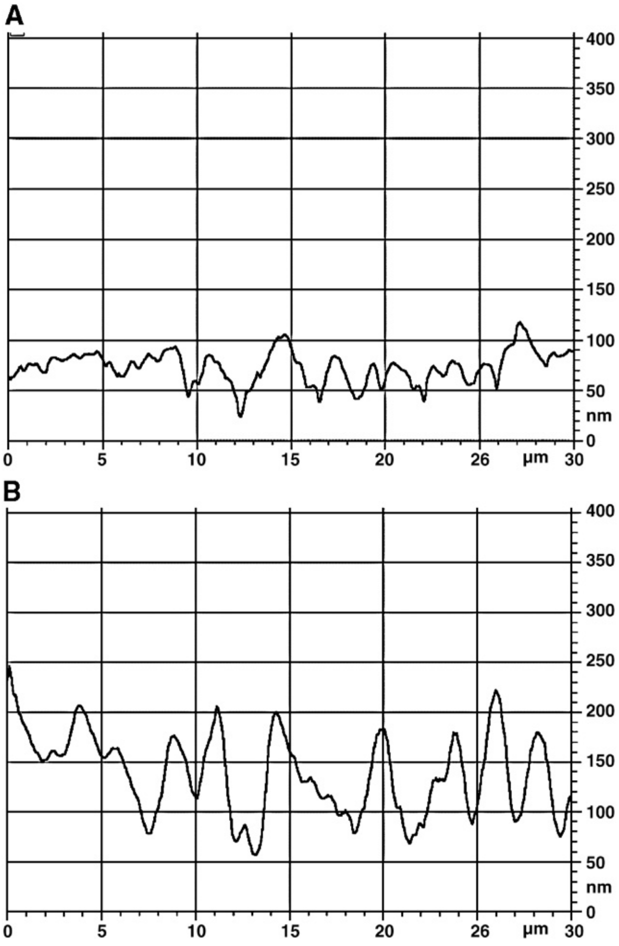

Fig. 2. Height profiles of the stainless-steel anode surface along white lines A in Fig. 1: (A) polished anode prior to the exposure by high-voltage electric pulses and (B) after the exposure to 120 exponential pulses with the amplitude of 4 kV and the duration of about 20 µs (dissolution charge $Q_{\mathrm{diss}}=0.24$ A s/cm^2).

It can be seen from these figures, that the surface of the polished stainless-steel electrode is rather smooth. Long straight narrow (width in the range of 1 µm) not deep (10–50 nm) scratches seen on the electrode surface are most likely a result of the mechanical polishing process. The average roughness, R_a, calculated for the anode surface three-dimensional image shown in Fig. 1A is 15 nm and the total roughness which is the vertical distance from the deepest valley to the highest peak, R_t, is 157 nm (Fig. 1A). Three-dimensional AFM images of the surface obtained in different electrode spots, give the total roughness of the surface in the range of 140–180 nm.

Then a series of the short exponential (time constant of about 20 µs) high-voltage (4 kV) electric pulses were applied to the chamber filled with the 154 mM sodium chloride solution and the surfaces of both electrodes (anode and cathode) were scanned by the atomic force microscope. A three-dimensional image of Fig. 1B provides quantitative information on the anode surface topography after the exposure to a series of 60 exponential electric pulses giving the total dissolution charge $Q_{\mathrm{diss}}=0.12$ A s/cm^2. As in the previous case, the scanning area was 30×30 µm^2.

It can be seen from Fig. 1B that the anode surface became much rougher. The average roughness of the anode surface, R_a, increased to 21 nm and the total roughness, R_t, to 274 nm (Fig. 1B). Three-dimensional AFM images of the surface obtained in different spots on the electrode, give the total roughness in the range of 250–300 nm. So, the roughness of the anode after the exposure to a series of 60 electric pulses has increased by 40–80%. These changes of the surface roughness were the result of the release of ions from the stainless-steel anode due to oxidation of the metals from which the anode was made (mainly iron, chromium, nickel, and manganese [29]). At the same time, no noticeable changes in the morphology of the cathode surface were detected (data not shown).

A great variety of the types and intensities of electric treatments is utilized in cell electromanipulation experiments. In the case of a single-pulse exposure, the dissolution charge varies from less than 0.001 A s/cm^2 [35] up to 0.2–0.3 A s/cm^2 [36–38]. To get closer to the upper limit of this range of a single-pulse dissolution charges, 120 exponential electric pulses with the amplitude of 4 kV, giving the total dissolution charge Q_{diss} = 0.24 A s/cm^2, were used. Because, in the case of a multi-pulse treatment, the release of metal ions from the anode proceeds in a cumulative manner [26], the use of a series of the pulses to get the required level of a dissolution charge is acceptable.

Then, the surface morphology of the anode was investigated. A three-dimensional image of the surface of the anode (Fig. 1C) shows that the total roughness of the surface, R_t, remarkably increased and reached 410 nm. The average roughness, R_a, increased to 47 nm. Three-dimensional AFM images of the surface obtained in different spots on the electrode, give the total roughness of the surface in the range of 380–450 nm.

The results presented here show that, under the anode dissolution conditions used here, the topography of the stainless-steel surface has been altered. Well-defined peaks (and valleys) with the width of 1–2 μm and the height of over 400 nm (Fig. 1C) have appeared. These peaks create local enhancements of the electric field at the interface between the solution and the electrode surface and can facilitate the occurrence of the electrical breakdown of the liquid samples. This reduces the maximum intensity of the electric field pulses that could be applied to the treatment chamber. Meanwhile, when using electroporation for the electroporative gene DNA transfer [19], microbial inactivation by pulsed electric fields [12], or other applications, often it is necessary to apply the pulses of as high electric field as possible [20]. As one of the possible solutions of this problem, polishing of the electrodes before use could be recommended. It was noticed earlier that this helps to avoid arcing at high voltages [15].

Our results reveal one more source of the possible inhomogeneity of an electric treatment of each cell — local electric field variations due to the roughness of the electrode surface created by strong electric pulses. Because the ultimate goal of some applications, such as electrochemotherapy and food pasteurization by pulsed electric fields, is to kill as many cells (tumor cells or microorganisms) as possible [21], each cell has to be exposed to an electric treatment of sufficient intensity and any inhomogeneity of an electric field is undesirable [21,22]. So, for preventing dielectric breakdown inside the treatment chamber and assuring the homogeneity of an electric treatment of each cell, the variations of the local electric field strength in the treatment chamber must be avoided [21–23]. It has been demonstrated that changing electrode orientation in the case of electrochemotherapy of solid tumors [21] or agitation of the liquid samples during microorganism inactivation by pulsed electric fields [22] allows to achieve more homogeneous electric treatment and improves the efficacy of electrochemotherapy and microorganism inactivation procedures [21,22].

The present work is the first demonstration that the use of the high-voltage electric pulses commonly utilized in cell electroporation procedures leads to the increase of the surface roughness of the stainless-steel anode, due to the dissolution of the anode material. This, in turn, can create local enhancements of the electric field which lead to the inhomogeneity of an electric treatment of each cell and facilitate the occurrence of the electrical breakdown of the liquid samples.

The authors hope, that the approach to study the changes of the surface morphology by using an AFM technique, utilized here and the results obtained can be helpful in finding the solutions for increasing the longevity of the electrodes as well as avoiding the problems arising due to the anodic half-reactions, such as ‘arcing’ and contamination of the solution by the release of metal ions from the electrodes.

Acknowledgment

This work was in part supported by grant FUNNANO (C-03046) from the Lithuanian State Science and Studies Foundation.

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