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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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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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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

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

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