Biosensors and Bioelectronics 24 (2009) 3600–3607

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Single cell and neural process experimentation using laterally applied electrical fields between pairs of closely apposed microelectrodes with vertical sidewalls

Wesley C. Chang*, David W. Sretavan

Departments of Ophthalmology and Physiology, Neuroscience and Bioengineering Programs, University of California, K110, Box 0730, UC San Francisco, 10 Koret Way, K-110, San Francisco, CA, 94143, USA

ARTICLE INFO

Article history:
Received 25 March 2009
Received in revised form 14 May 2009
Accepted 19 May 2009
Available online 27 May 2009

Keywords:
Microelectrode array
Neurons
Axon
Electroporation
Electrodes with vertical sidewalls
Field stimulation

ABSTRACT

As biomedical research has moved increasingly towards experimentation on single cells and subcellular structures, there has been a need for microscale devices that can perform manipulation and stimulation at a correspondingly small scale. We propose a microelectrode array (MEA) featuring thickened microelectrodes with vertical sidewalls (VSW) to focus electrical fields horizontally on targets positioned in between paired electrodes. These microelectrodes were fabricated using gold electroplating that was molded by photolithographically patterned SU-8 photoresist. Finite element modeling showed that paired VSW electrodes produce more uniform electrical fields compared to conventional planar microelectrodes. Using paired microelectrodes, 3 μm thick and spaced 10 μm apart, we were able to perform local electroporation of individual axonal processes, as demonstrated by entry of EGTA to locally chelate intra-axonal calcium, quenching the fluorescence of a pre-loaded calcium indicator dye. The same electrode configuration was used to electroporate individual cells, resulting in the targeted transfection of a transgene expressing a cytoplasmically soluble green fluorescent protein (GFP). In addition to electropration, our electrode configuration was also capable of precisely targeted field stimulation on individual neurons, resulting in action potentials that could be tracked by optical means. With its ability to deliver well-characterized electrical fields and its versatility, our configuration of paired VSW electrodes may provide the basis for a new tool for high-throughput and high-content experimentation in broad areas of neuroscience and biomedical research.

1. Introduction

In recent years, there has been growing interest in developing experimental methods to study single cells in order to probe deeper into intracellular events and to understand variations among individual cells and not be limited by the averaging effects from studying whole populations of cells (Andersson and van den Berg, 2004). Single cell analyses benefit from methods in which different cells on a single platform can be individually subjected to widely varying conditions and observed. At smaller scales, there is even need for localized stimulation or delivery of material into specialized, sub-cellular structures. The new experimental techniques at the microscale have come to rely in part on the various functions performed by microelectrodes. Historically, the use of microelectrodes to deliver localized electrical fields to individual cells has yielded many proven applications in basic biomedical research and in drug discovery (Albrecht et al., 2004; Huang et al., 2007; Jain and Muthuswamy, 2007; Lin et al., 2004; Pearce and Williams, 2007; Pine, 2006; Rajaraman et al., 2007; Ravula et al., 2006; Stett et al., 2003; Voldman et al., 2002). Microelectrodes have typically been used to stimulate and record signals from electrically active cells, such as neurons and muscle cells (Ravula et al., 2006; Stett et al., 2003; Taketani and Baudry, 2006). However, microelectrodes have also been used to transiently open cell membranes via electroporation to deliver normally impermeant materials, such as macromolecules and genetic material, into cells (Chang et al., 1992; Fox et al., 2006; Huang et al., 2007; Jain and Muthuswamy, 2007; Lee et al., 2006; Lin et al., 2004; Olofsson et al., 2003; Yuan, 2007). More recently, AC electrical fields have been used to spatially manipulate or trap cells based on the principle of dielectrophoresis (Albrecht et al., 2004; Gascoyne and Vykoukal, 2002; Voldman et al., 2002; Wang et al., 2007).

Microelectrodes are now routinely produced in array formats using techniques from microelectronic fabrication (Pearce and Williams, 2007; Stett et al., 2003; Taketani and Baudry, 2006). The basic construction of such microelectrodes consists of planar metal films lithographically patterned and etched on a planar glass substrate. However, a drawback of these planar electrodes is the high non-uniformity of electric fields induced along the edges of these electrodes (Wang et al., 2007). To circumvent this fundamental

* Corresponding author. Tel.: +1 415 476 4135.
E-mail address: changw@vision.ucsf.edu (W.C. Chang).

0956-5663/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2009.05.024

disadvantage, alternative electrode configurations have been devised to reduce field non-uniformities. In particular, the fabrication of thickened, three-dimensional electrodes eliminates sharp edges along the substrate while also reducing the impedances between electrode and sample (Barbier et al., 2006; Gross et al., 2002; Heuschkel et al., 2006; Rajaraman et al., 2007; Thiebaud et al., 1997; Voldman et al., 2002; Wang et al., 2007). These raised structures can range from simple "hillocks" (Thiebaud et al., 1997) or etched conical structures (Heuschkel et al., 2006) to specifically molded electrodes shaped like posts, pillars, or blocks rising vertically from a substrate, which could then be integrated with various microfluidic devices (Rajaraman et al., 2007; Voldman et al., 2002; Wang et al., 2007). Designs involving these specifically shaped electrodes have taken advantage of photolithographically patterned photoresist to serve as molds to precisely shape the deposition of metal by electroplating (Rajaraman et al., 2007; Song and Ajmera, 2003; Voldman et al., 2002; Wang et al., 2007). Electrodes with smooth, vertical sidewalls have been produced using this method, and some investigators have used these electrodes to apply electrical fields horizontally, with biological samples positioned in the specific target region between electrode sidewalls. This use of laterally applied electrical fields from vertical sidewalls provides a greater spatial uniformity in field strength and also confers an important advantage for imaging, since the cells or subcellular structures of interest are positioned in between and not obscured by the electrodes. So far, this concept for microelectrode design has only been used on cells freely suspended in fluid media, and the spacing between electrodes have been much larger than the individual cells (Voldman et al., 2002; Wang et al., 2007).

In this study, we present a refined method of making paired vertical sidewall (VSW) electrodes that permits sidewalls to be closely apposed to direct uniform electrical fields in a confined volume in order to exclusively target individual cells and even subcellular processes. We focused on adherent cells and their cellular processes since many cell types require adhesion to a substrate in order to differentiate, polarize, function, and survive. We paid particular attention to neurons, because they are electrically active cells and are highly polarized, projecting long axons, which are highly specialized sub-cellular structures essential for neuronal function. In this study, we demonstrate the capability of paired VSW electrodes for experimentation on isolated individual axons within a dense neuronal field and to direct electrical fields specifically to these subcellular structures to perform focused field stimulation of individual axons and localized, axonal electroporation for delivery of reagents. On the scale of whole cells, we also demonstrate that paired VSW electrodes can likewise focus electroporation to an individual cell from amongst a large population to perform targeted gene transfection.

2. Methods

2.1. Finite element modeling

To illustrate the benefits of using electrical fields applied laterally between two closely spaced vertical sidewall electrodes, finite element modeling of the steady-state electrical field in the region between electrodes on glass and immersed in low-conductivity media was performed using COMSOL Multiphysics. We compared the electrical fields generated by a pair of planar electrodes with the fields generated by a pair of raised, three-dimensional VSW electrodes (Fig. 1, S1). For this demonstration, the voltages along the surface of opposing electrodes were held to explicit potentials of 1 and 0 V, respectively. The modeling was performed in a two-dimensional environment and depicted the vertical cross-sections of the opposing electrodes. For each type of electrode (planar vs. VSW), the induction of transmembrane potentials in axonal processes of neurons was represented in two separate situations by: (1) positioning a circular cross-section (representing an axon) midway in between electrodes; or (2) in a separate simulation, by positioning the axon immediately adjacent to one of the electrodes (Fig. S1 in Supplementary material).

2.2. Electrode fabrication

The fabrication of VSW electrodes was based on previous methods for creating raised gold microelectrodes (Song and Ajmera, 2003; Voldman et al., 2002; Wang et al., 2007) but also included important augmentations to the fabrication process to produce paired microelectrodes that were not only shaped with vertical sidewalls but also positioned in close apposition, separated by only 10 μm. Briefly, a foundation of titanium and gold was deposited via ion beam evaporation on clean pyrex wafers. The first layer of photolithography patterned the gold film to serve as the outline for both the raised electrodes and planar electrical traces. In the second lithographic step, SU-8 25 (MicroChem Corp.) was spin-coated onto the wafer, and the footprint for the raised electrodes was then

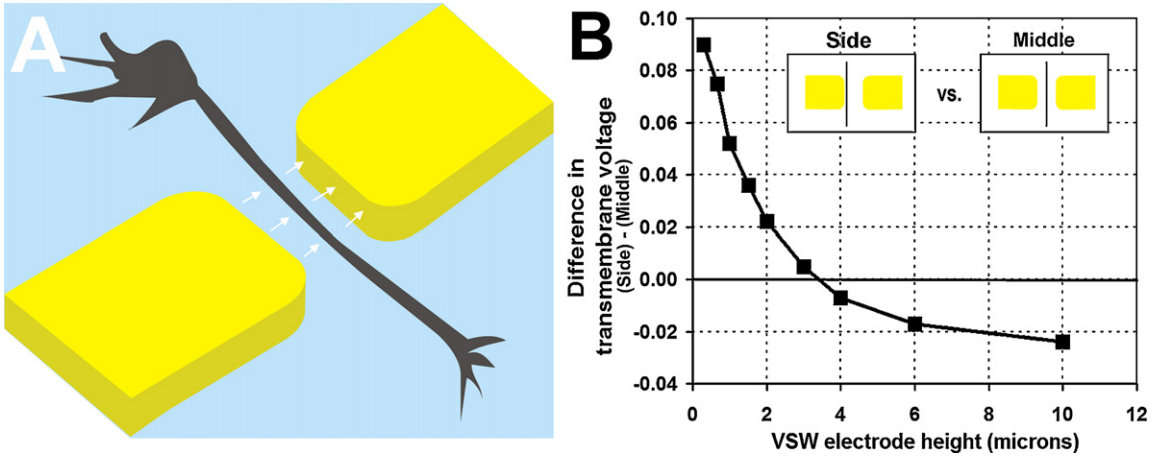

Fig. 1. (A) An axon (dark grey) projecting into a 10 μm gap between a VSW electrode pair (gold). The application of electrical fields (white arrows) in between these electrodes induces a transmembrane potential in the axon segment within the gap. (B) The transmembrane potential induced on the axon in (A) depends on where it is positioned in proximity to an electrode. This plot shows the difference in the induced transmembrane potential for the two limiting cases (shown in the inset) of (1) the axon positioned midway between electrodes and; (2) the axon positioned immediately adjacent to an electrode vs. electrode thickness. This difference is minimized when the VSW electrode is approximately 3 μm thick.