

A NOVEL SYSTEM FOR MEASUREMENT OF ELECTROPHYSIOLOGICAL SIGNALS ASSOCIATED WITH TRAUMATIC NEURONAL INJURY

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ABSTRACT

The cellular and electrophysiological events that occur during and immediately after a traumatic insult to neurons are poorly understood. In previous studies, patch clamp recordings have been used to characterize stretch-induced delayed depolarization [1]; however, this technique only allows for the investigation of a single neuron and prevents examination until after injury has taken place. To address these problems, we have designed and manufactured a microanalysis system that integrates a cell shearing injury device and a multi-electrode array (MEA), making it possible to assess functionality of a neural network before, during, and following mechanical insult.

KEYWORDS

Multi-electrode Array, Traumatic Brain Injury, Electrophysiology, Rat Neurons

INTRODUCTION

During a traumatic insult, brain tissue is subjected to stresses and strains at high rates and magnitudes, which surpass cellular threshold and lead to cell dysfunction or death [2]. Our cell shearing injury electrophysiological recording device produces a mechanical perturbation that models the high rates of stresses present during traumatic brain injury (TBI). This *in vitro* model of traumatic brain injury, coupled with real time extracellular recording capabilities, allows us to evaluate how neuronal network dynamics are affected by injury. A multi-electrode array is well suited for this application as it allows for the continuous and non-invasive monitoring of cells. The recorded changes in electrical signaling will offer new insights into neuronal functional recovery following insult. To our knowledge, this is the first demonstration of an electrophysiological recording device capable of studying the effects of shear stress deformation for a network of neurons.

DESIGN AND PERFORMANCE

This cellular analysis tool is comprised of two main components—the multi-electrode array and the cell shearing injury device. The fabrication of the MEA component, which

employs only two masks, uses conventional surface micromachining technology (Figure 1). Photoresist is patterned to define the MEA wiring. Layers of titanium and gold are sequentially e-beam evaporated with the undesired portions lifted off in acetone. SU-8 is patterned to form the openings to the contact pads, and electrodes. The SU-8 serves as both the electroplating mold for the platinum black electrodes and as the final insulation layer. Platinum black electrodes are electroplated into the SU-8 molds under ultrasonic conditions which effectively removes loosely adherent platinum deposits to insure long lasting adhesion [3]. The thick SU-8 insulator, coupled with the low impedance of the platinum electrodes, provides an excellent signal-to-noise ratio.

The injury MEA, or “iMEA”, which couples the cell shearing injury device and MEA, work as follows: A glass cone rotates above a planar MEA, to which two dimensional

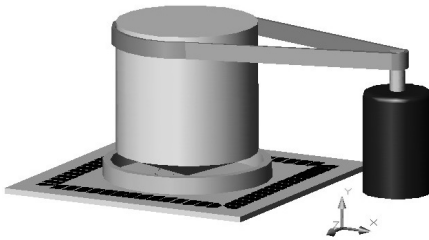


Figure 2: Illustration of the fundamental principle of operation. A shearing cone is precisely controlled by a servo motor and a computer (not shown) to simulate the main type of deformation during Traumatic brain Injury. The electrode array records neural signaling before, during, and after injury

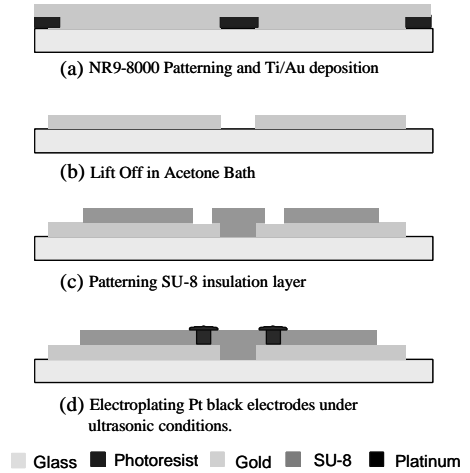


Figure 1: Fabrication sequence for the MEA component

cultures are adhered (Figure 2). The rotation of the cone induces a velocity gradient perpendicular to the cell plate in the fluid that fills the gap between the cone and MEA. A computer-controlled servo motor precisely controls the shear stress (up to 1200 dynes/cm²) and shear rate (with rise times as short as 20ms) that is applied to the cultures. Meanwhile, the electrode array records neural signals through a commercial data acquisition system (Figure 3). The electrodes, which are optimally designed for maximal signal-to- noise ratio, are centered between the apex and boundary of the cone. The cone geometry yields uniform

shear stress throughout the plate[4]. MEA cell plating has been achieved and is performed according to a protocol modified from [5].

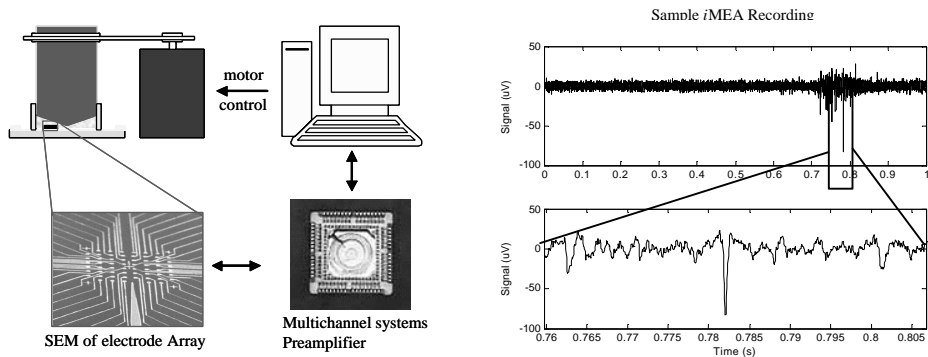


Figure 3: (Left) Illustration of the *i*MEA system. The computer controls the shearing force via the servo motor while recording from electrodes on the MEA. (Right) Recording from day 10, rat cortical neurons using the *i*MEA system.

CONCLUSION

Incorporation of a neuronal cell shearing injury device with a planar array of micro-fabricated electrodes has been introduced. The system allows for the electrophysiological investigation of a network of neurons before, during and after injury. E18 primary cortical neurons have been successfully cultured on the MEA, and electrophysiological signals have been acquired. Additionally, controlled cell injury has been achieved. Continued investigation of electrophysiological changes may elucidate the complex cellular pathways associated with traumatic brain injury.

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