

Multielectrode Arrays Restoration Using 3D Printing Technologies

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Scientific and technological achievements became intensively used on the practice when their application became economically efficient. In this study, we demonstrated the potential of using filament 3D printing technologies for repairing multielectrode arrays (MEAs), a key expensive component of most in vitro bioelectronic devices. We present a method for restoring the functionality of MEAs after its reference (counting) electrode failure by replacing them with a special 3D printed holder equipped with a tiny platinum strip. The obtained results can be used for an extension of the life cycle of MEAs, making the applications of bioelectronic technologies more accessible for solving actual problems in medicine and biology.

Keywords: multielectrode arrays, impedance spectroscopy, 3D printing, repair.

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The success of practical application of scientific novelties is determined not least by their economic efficiency. Among the examples of inventions noted for their economic merits are the sun valve [1], integrated circuits [2], and heterojunction light sources [3,4]. At the same time, bioelectronics [5–8], which is one of the rapidly developing interdisciplinary fields of science, is presently faced with the problem of high cost of manufacture and usage of the unique equipment and expensive materials. For example, breakthrough research projects in the field of visual prosthetics at Second Sight Inc. were terminated due to the unprofitability of the used technologies [9], and commercial cytosensory interfaces (so-called multielectrode arrays, MEAs) from MultiChannels Systems, Panasonic, ACEA BioScience, and other companies are expensive and unreliable. Specifically, one of the main causes of MEA failure is damage of the reference electrodes (REs, also known as counting electrodes) as a result of their burnout, mechanical scratching during cleaning of MEAs from cells, or loss of contact between the RE and external pads due to chipping of the MEA glass substrate. REs are used to close the electric circuit of the working electrodes, and they are normally located along the periphery of the bottom of a MEA and also have a larger area than its working electrodes. Owing to this, they are used to close the electric circuit of the working electrodes. Thus, REs are an essential part of an MEA, and it is economically and technologically important to ensure their serviceability. This is the reason why we attempted to solve the above-mentioned problem of increasing the economical efficiency of bioelectronic devices by devising a method for restoring the functionality of MEAs with damaged reference electrodes. A sample with chipped external contacts for reference electrodes was chosen as a test MEA service case. An external platinum

reference electrode, which is mounted in an MEA Petri dish using an ergonomic 3D-printed console bracket, was fabricated for it. The characteristics of the resulting RE are on par with those of the original reference electrodes of the MEA, allowing one to take full advantage of the serviced MEA in electrophysiological studies.

Blender 4.3.0 was used to design a 3D model of an external reference electrode system for commercial MEAs. A Flying Bear S1 (Zhejiang FlyingBear 3D Technology Co., Ltd, China) printer was used for 3D printing. The proposed external RE system for the commercial MEAs consisted of a special bracket (Figs. 1, *a* and *b*; the STL model is available online [10]) made of PLA 3D plastic (Guangzhou Zhiwen Technology, China, Fig. 1, *a*) and of biocompatible platinum strip $2 \times 15 \times 0.2 \text{ mm}^3$ in size. Unlike the classical silver chloride wire electrode, the resulting structure provides an opportunity for prototyping of implantable devices [11]. The RE was assembled by attaching the platinum strip to the printed bracket over a pre-mounted angled plug using two pieces of heat-shrinkable tubing (Figs. 1, *a* and *b*). A pressure electrical contact was thus established between the plug and the plate. The plate was then bent carefully into the shape of the \int symbol to form a smooth tip that would not scratch the MEA bottom. The angled plug provided mechanical connection of wires to the RE (Fig. 1, *c*). A 3D-printed holder was used to connect the MEA to the external RE (Fig. 1, *c*). The RE was placed onto holder with a bolt and a nut recessed into the holder.

The performance of the fabricated reference electrode was tested using impedance spectroscopy with an MED P5155 MEA (MED64, Panasonic, Japan; transparent ITO electrodes with removed platinum coating), which had one of the four REs ($180 \times 180 \mu\text{m}^2$) damaged as a result of chipping of its contact pad (Fig. 1, *a*). This design

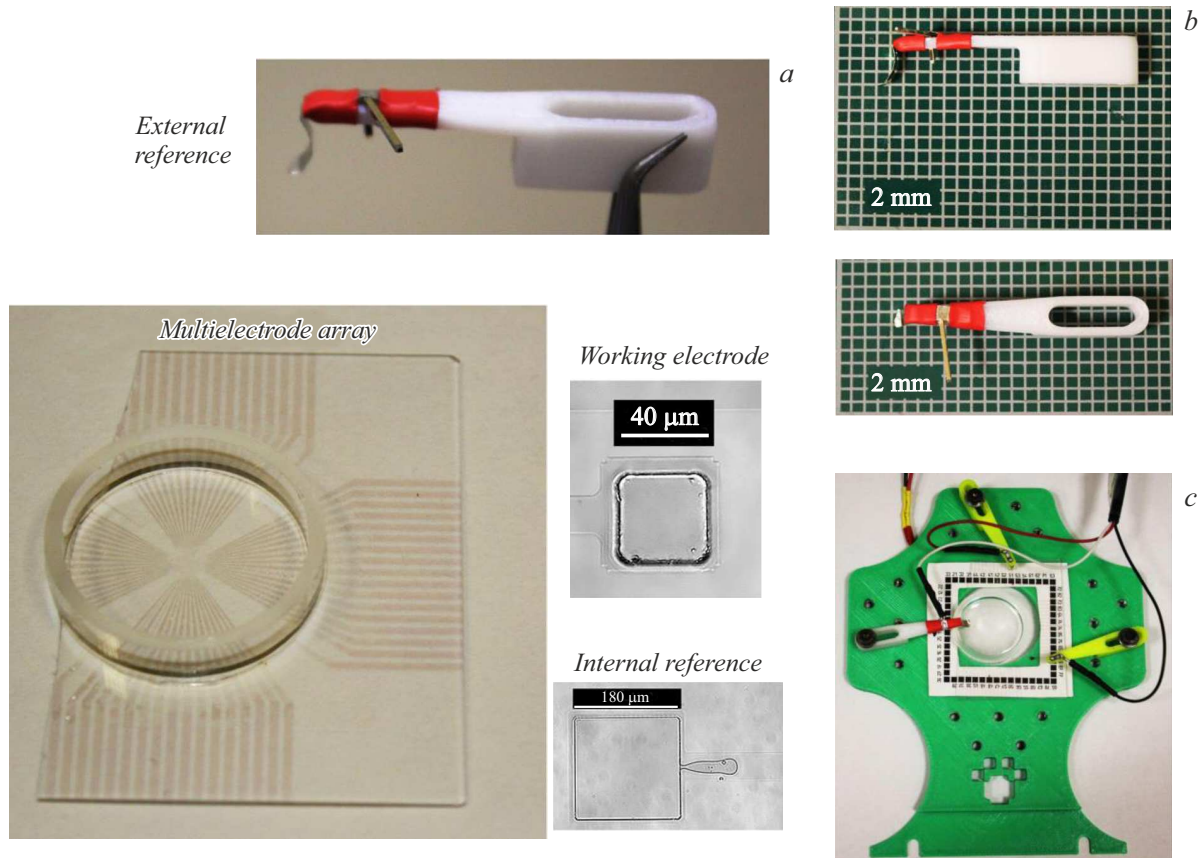


Figure 1. Materials for testing the external RE. *a* — Photographic images of an MED P5155 multielectrode array with its left part, which contained the contact pad for the internal RE, chipped off (left down); its working electrode and the internal RE (grayscale photos); and the external RE fabricated in this study (upper image). *b* — Photographic images of the external RE on a scale grid. *c* — Photographic image of the external RE mounted on the MEA and an example of its connection to external electronic components.

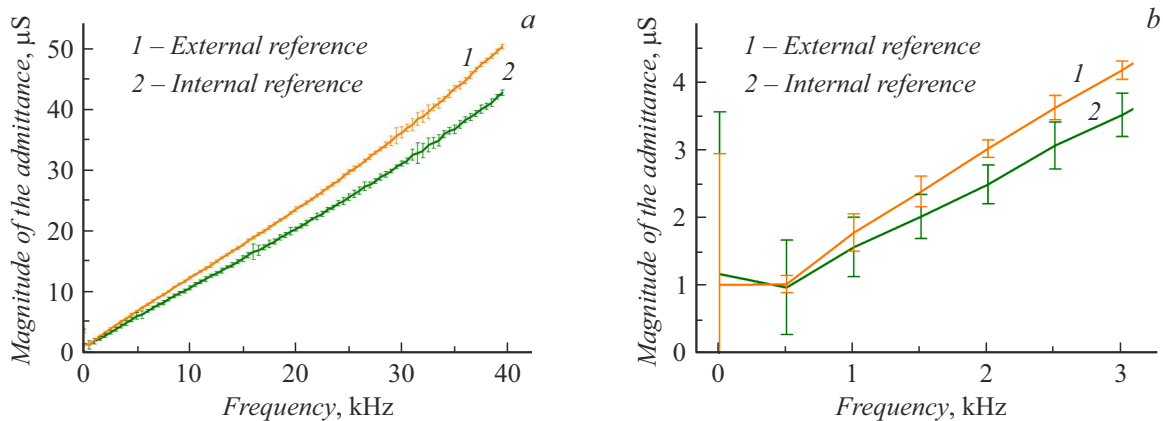


Figure 2. Comparison of the admittance spectra of the working MEA electrode measured relative to the external and internal REs within the entire frequency range (*a*) and at low frequencies (*b*). Errors correspond to 99.9% confidence intervals.

of the experiment allowed us to demonstrate clearly the performance capacity of the fabricated RE and compare its characteristics with those of the internal REs of MED P5155. The impedance was measured relative to one of the working MEA microelectrodes ($40 \times 40 \mu\text{m}^2$) using the setup detailed in [12] within the frequency range of 2 Hz–40 kHz with a step of 500 Hz and a probing voltage

amplitude of 15 mV in a phosphate-buffered saline solution (Biolot, Russia).

Photographic images of the manufactured RE are presented in Figs. 1, *a* and *b*. Figures 1, *a* and *c* also show the damaged MEA and the proposed RE mounted on it, respectively. It follows from the obtained data that the proposed RE design is compatible with standard-size

commercial MEAs and is easy to mount. The admittance analysis data presented in Fig. 2 demonstrate that the macroscopic external RE does not degrade the electrical properties of MEA electrodes. Specifically, the admittance spectra obtained with two RE types are statistically indistinguishable within the low-frequency range (up to 1.5 kHz); at higher frequencies, the admittance measured relative to the external RE is several percent higher than the admittance measured relative to the internal one, which is attributable to the fact that the external RE is larger in area. This result may be used in practice to improve the signal-to-noise ratio in impedance and electrophysiological studies with MEAs with micrometer-scale REs. It should also be noted that REs are macroscopic MEA parts; therefore, it is much simpler and cheaper to fabricate them than to produce a new MEA, which requires lithographic processing [13] and expensive ITO deposition equipment [14].

Thus, in this study the potential of 3D printing based servicing of damaged reference electrodes of commercial MEAs was demonstrated. Specifically, a 3D model of a bracket holder for a platinum strip, which is immersed in an MEA Petri dish and replaces a damaged RE, was proposed. The results of testing the obtained RE in restoration of a commercial MED P5155 array revealed that the admittance of its working electrode measured relative to the 3D printing based RE is comparable to (at low frequencies up to 1.5 kHz) or several percent higher (at high frequencies, 40 kHz) than the admittance measured relative to the internal MEA RE. Therefore, the obtained data show that the proposed technology for restoration of MEAs may be used both to restore their functionality and to increase the signal-to-noise ratio. We hope that the results of this study will not only help reduce the cost of use of MEAs in medical and biological practice, but will also pave the way for designing more advanced bioelectronic devices aimed at solving a wide range of biosensor engineering problems.

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Conflict of interest

The authors declare that they have no conflict of interest.

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