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Autostainer feature for multielectrode arrays: proof-of-concept

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Abstract. Today, bioelectronics technologies are opening new horizons and paving the way for future development using a wide range of materials. For example, numerous purely electronic materials, such as silicon, textolite and various polymer masks, are very promising for the purposes of cellular bioelectronics in vitro, but their optical properties limit their use, since modern microscopic control is still the most reliable tool for live cell diagnostics. This problem can be solved with the help of special dyes, however the staining procedure requires reagents and time, which on an industrial scale will lead to costs and a slowdown in the production process. Here we propose a solution to this issue based on the fabrication of cell-based bioelectronic devices that automatically stain cells without any additional sample manipulation.

Keywords: MEA, autostainer, Hoechst, silicon, PETG

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Автоматическая покраска клеток в мультиэлектродных матрицах: пилотное исследование

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Аннотация. Сегодня в направлении биоэлектроники (БиоЭл) открываются все новые горизонты благодаря внедрению в ее технологии последних достижений из области материаловедения. Тем не менее, ряд перспективных для БиоЭл материалов, таких как кремний, текстолит и различные полимерные маски, до сих пор применяются в ней ограниченно для *in vitro* исследований, поскольку такие материалы непрозрачны и поэтому несовместимы с классической микроскопической диагностикой клеток в режиме проходящего света. Данную проблему можно решить с помощью использования специальных красителей, однако сама процедура покраски клеток достаточно

трудозатратна и в промышленных масштабах экономически неэффективна. В настоящей работе мы предлагаем решение вышеочерченной проблемы, которое заключается во внедрении в БиоЭл приборы блока автоматической покраски клеток.

Ключевые слова: мультиэлектродные матрицы, Hoechst, кремний, PETG

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Introduction

The bioelectronics (BE) industry today covers a wide range of applications such as biosensing, neuroprosthetics, laboratory disease monitoring, living cell research *in vitro* etc [1–3]. One of the challenges facing BE device development is choice of materials that must be biocompatible, inexpensive, easy to usage, and compatible with modern biocharacterization methods. For these reasons, some promising electronic industry materials, such as the classical semiconductors silicon and germanium or insulators such as textolite, are today used much less frequently in cellular biosensors than glass or transparent polymers, due to their incompatibility with microscopic transfer-light techniques that are usually utilized to check the number of cells and their state. Thus, to include opaque materials in the BE arsenal, it is necessary to use staining methods that require time, reagent consumption, and additional manipulations [4] with the cell, i.e. this approach is invasive and economically ineffective.

One possible solution to this problem is to simplify or eliminate the stage of manipulation with cell staining through the use of specially prepared additional parts of the BE device. Here we propose to use the second approach, which is very useful for BE devices such as multielectrode arrays (MEAs). Each MEA consists of a substrate with planar electrodes and a Petri dish ring, the function of which is typically to maintain the cellular medium on the MEA electrodes. However, we can increase the functionality of such a ring by treating it with highly adhesive dyes such as Hoechst [5]. Thus, the ring treated in this way will become a source of dye, and cells cultured with it in MEA will be automatically stained without any additional cell preparation steps. Therefore, in this article, we will provide a proof-of-concept of this approach to create an MEA with autostaining feature.

Materials and Methods

To test the ring auto-staining technique, we assembled an MEA-like device from silicon wafer [Si (100) doped with sulfur, surface coated with natural SiO_2], PDMS Sylgard 184 (DowCorning, Germany, 10/1 PDMS/hardening agent ratio), and a 3D-printed ring of polyethylene terephthalate glycol (PETG). The technological process is shown in Fig. 1. First, the surface of the silicon and rings was cleaned with ethanol and dried with compressed air. A small amount of PDMS was then applied to the bottom of the rings, after which silicon chips were glued to them (Fig. 1,a). Next, the resulting structures were baked at a temperature of 65 °C for 30 minutes in a bottom-up position (Fig. 1, *b*). Such manipulations (small amount of PDMS and bottom-up baking) are necessary to prevent leakage of PDMS onto the MEA prototype dish bottom. It should also be emphasized that the use of PDMS at this stage is aimed at ensuring a sealed contact between the silicon substrate and the ring, and not at creating a strong bond between them. After the first hardening step, PDMS was applied a second time to the outer part of the ring to ensure a strong connection between the ring and the substrate, after which the sample was hardened again at a temperature of 65 °C for 30 minutes (Fig. 1, *c*).

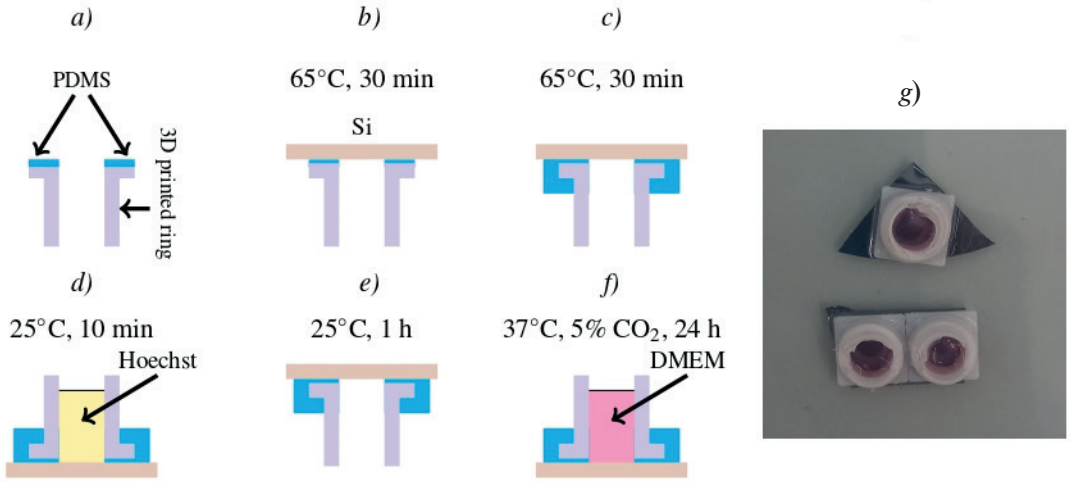

Fig. 1. MEA prototype with autostainer feature fabrication process. PDMS deposition (*a*), formation of the hermetic contact between silicon and ring (*b*), formation of the strength joint between silicon and ring (*c*), exposing device by Hoechst (*d*), washing and drying MEA prototype (*e*), seeding and culturing cells in MEA prototype (*f*), top-view photograph of the obtained MEA-like devices (*g*)

The resulting prototype MEA was then washed with soap and water, rinsed with deionized water, and sterilised with ethanol. Finally, a Hoechst solution (ThermoFisher, USA, 1:1000 dilution in PBS) was added to the obtained MEA prototype for 10 min (Fig. 1, *d*), after which the sample was washed, sterilised again and dried in a bottom-up position (Fig. 1, *e*). For the control sample of the MEA prototype, the step shown in (Fig. 1, *d*) (Hoechst processing) has been omitted. The external view of the obtained devices is shown on (Fig. 1, *g*). As a sample for autostainer's test the HeLa cells were used. The cells were obtained from bank of the cell cultures from Institute of Cytology RAS. One day before experiment the cells were seeded onto MEA prototypes and cultivated at 37 °C and 5% CO_2 in DMEM medium (Biolot, Russia) with 5% FBS (Sigma Alrich, USA) and 1.5% gentamicine (Biolot, Russia) (Fig. 1, *f*). Immediately before experiment the cells' medium was replaced by PBS with propidium iodide dye (PI, ThermoFisher Scientific, USA) for checking cells viability. The microphotographs of the cells were made using Leica 4000 DM B fluorescence microscope (Leica, Germany).

Results and Discussion

The results obtained from testing the autostainer-MEA prototype are presented in Fig. 2. It can be seen that control (Fig. 2, *a*) and autostaining samples (Fig. 2, *b*, *d*, *e*, *f*) allow diagnosing cells in the non-fluorescent mode of bright reflected light in the case when the microscope lens looks at the center of the Petri dish, where optical distortions caused by the meniscus of the cellular medium are minimal. It should also be noted that the cell viability of all samples is high (Fig. 2, PI channel). However, the UV-pumped Hoechst-images (Fig. 2, *a*) and (Fig. 2, *b-f*) are significantly different. More precisely, the control sample (Fig. 2, *a*) not treated with Hoechst dye exhibits a weak cell autofluorescence signal, making it very difficult to isolate individual cells.

In contrast, cells incubated in the Hoechst-treated device (Fig. 2, *b-f*) showed very clear nuclear fluorescence, which can be used to count cells and determine their location.

Looking at the brightfield images Fig. 2, one might get the impression that the use of fluorescent dyes is not necessary. However, this misconception immediately disappears in the case of studying cells located in the immediate vicinity of the edge of the Petri dish ring (Fig. 2, *c*). Indeed, one of the significant but not obvious differences between microscopic examination of cells on transparent substrates (where the microscope lens can be placed *under a flat* Petri dish) and opaque substrates (where the lens must be placed *above* the Petri dish) is the existence of a meniscus near the rings border of the Petri dish, which causes optical distortion of the microscopic top-view image (see left photograph on Fig. 2, *c*). This effect reduces photographs resolution due to optical smoothing of the image, resulting in the inability to distinguish individual cells within populations using reflected light alone.

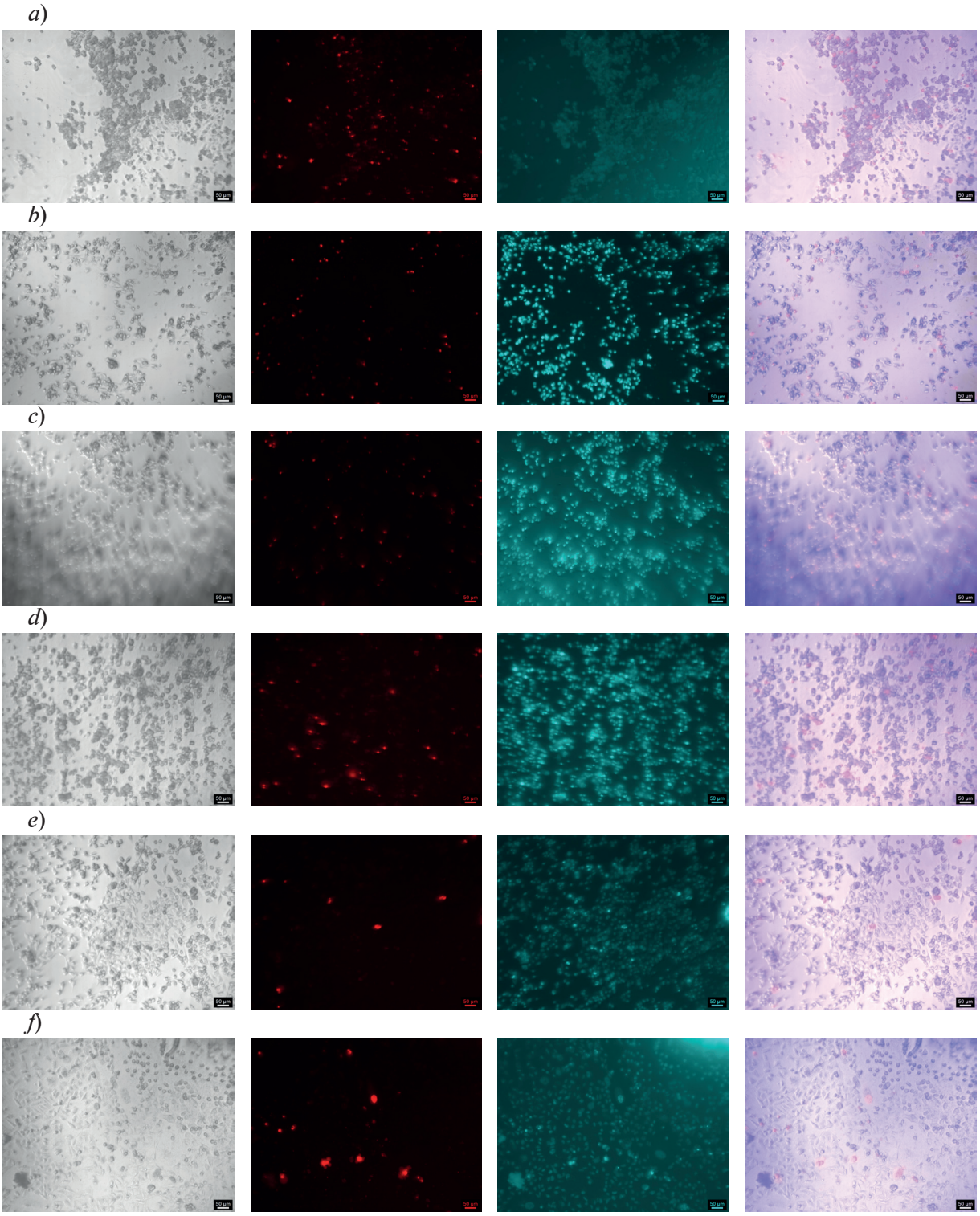

Fig. 2. Demonstration of the auto-staining effect. A photograph of the control MEA prototype is shown in panels (*a*), and other panels corresponds to MEA prototype with autostaining function as follows: (*b*) and (*c*) shows MEA after 2 washing/cells seeding procedures, (*d*, *e*, *f*) corresponds to 3, 4, and 5 washing/cells seeding cycles, respectively. Scalebar corresponds to 50 μm

For MEAs using the Lab-on-Chip concept, this effect is very negative because when developing such devices, engineers try to use the entire available area of the Petri dish, and the inability to monitoring the electrodes near the Petri dish ring can lead to the loss of part or all of the biosignal.

The clear advantage of using fluorescent dyes is shown in Fig. 2, *c*), from which it can be seen that near the edge of the ring the brightfield image is completely distorted, but the fluorescent channels still work perfectly, transmitting information about the number of cells and their position. This phenomenon can be explained by the selective staining property of dyes. Because cell nuclei are smaller than a whole cell, and therefore the distance between nuclei is greater than the distance between cells, the drop in resolution caused by meniscus optical distortion does not affect their images as significantly as on bright-field images of cells, which resulting in sharpness nuclei photographs in fluorescence mode. During experiments we also observed that silicon MEA with PETG ring conserve autostaining effect up to five cells seeding procedures.

Conclusion

In this paper, we demonstrated how the autostaining feature can be implemented in MEA devices and how it can be used to monitor cells *in vitro* in opaque MEAs. More precisely, we showed that MEAs pretreated with Hoechst dye, even after five wash cycles, still stain cell nuclei without any additional manipulation by the MEA user. We also demonstrated the advantage of our autostaining method over brightfield methods by taking photographs near the edge of the MEA, where brightfield methods are not applicable due to image distortion caused by meniscus, but the fluorescence methods in such case still works correctly. We believe that the results of our work open up new prospects for the creation of bioelectronic devices from modern electronic opaque materials, which will increase the versatility of such devices and, thereby, allow solving current problems in biosensing, healthcare and cytology.

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REFERENCES

1. **Stupin D. Daniil et. al.,** Bioimpedance spectroscopy: basics and applications, ACS Biomaterials Science & Engineering. (7) (6) (2021) 1962–1986.

2. **Neroev V.V., Astakhov Yu.S., Lobanova M.M., Stupin D.D., et. al.,** Artificial vision: advances, topical issues, and prospects, Russian Ophthalmological Journal. (11) (3) (2018) 3–27.

3. **Jiang Wen G., ed.,** Electric cell-substrate impedance sensing and cancer metastasis. Springer Science & Business Media. (17) 2012.

4. **Matsumoto B.,** Cell biological applications of confocal microscopy. Elsevier. (70) 2003.

5. **Bucevičius J., Lukinavičius G., Gerasimaitė R.,** The use of hoechst dyes for DNA staining and beyond. Chemosensors. (6.2) (2018) 18.

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