New Fabrication Method for M-Gripper to Assess Interface Stability

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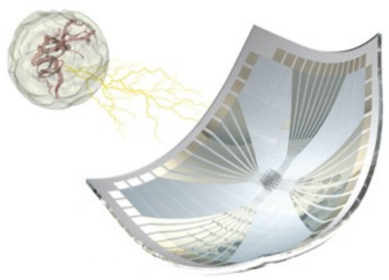
Introduction



Organoids, as advanced cell models, replicate in vivo cellular processes and have revolutionized our understanding of various biological phenomena, including tumor biology and neuronal disorders. These three-dimensional structures mimic the complex architecture and functionality of human tissues, offering a promising platform for studying disease mechanisms and developing novel therapeutic strategies.

Traditional sensors like patch clamps and multielectrode arrays (MEA) have been essential in studying brain organoids but have significant limitations. These methods typically capture activities only from the bottom surface of brain organoids, restricting data comprehensiveness. Despite modifications, such as incorporating 3D nanostructures to enhance signal strength and achieve a higher signal-to-noise ratio, the recording area remains confined and does not fully exploit the organoids' three-dimensional nature.

To overcome these limitations, advanced organoid sensors with long-term stable electrical interfaces are necessary. An innovative solution is the "M-Gripper," a new 3D-organoid sensor manipulated by a magnetic field to self-fold and avoid unnecessary contact with organoids. This design eliminates the need for wired physical apparatus, reducing potential interference and damage to the delicate organoid structures. The M-Gripper's efficacy depends on its ability to establish a good interface with the organoid, producing high-resolution images and accurate data. In this study, fluorescence imaging will be used to evaluate this interface, ensuring a stable and effective connection. The device was fabricated using lithography, with conditions optimized for fluorescent dye.

Experimental Methods

Optimization of patternable polymer and dye

1) Determination of the type of polymer and fluorescent dye

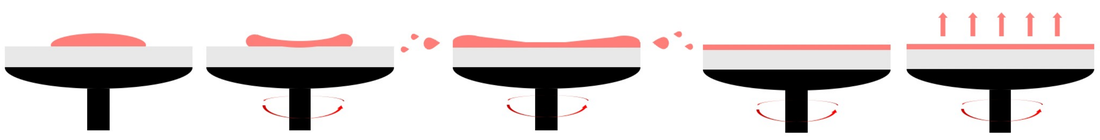
SU8 is negative photoresist and commonly used, as it is easily patternable and thickness controllable photoresistor. In addition, by its biocompatible character, it is ideal in the purpose of BioMems. Accordingly, SU8 is used in this study.

A study to determine the most suitable fluorescent dye to use with SU8 was conducted on various candidate dyes, Rhodamine-B (RhB), Coumarin-540A (C540A), Cibacron-Yellow (CBY), Fluorescein (FL), Pyrromethene (Py580), and Red light-emitting spiro copolymer (RLSC). The study concluded that Rhodamine B(RhB) was the most suitable fluorescent dye for use with SU8.

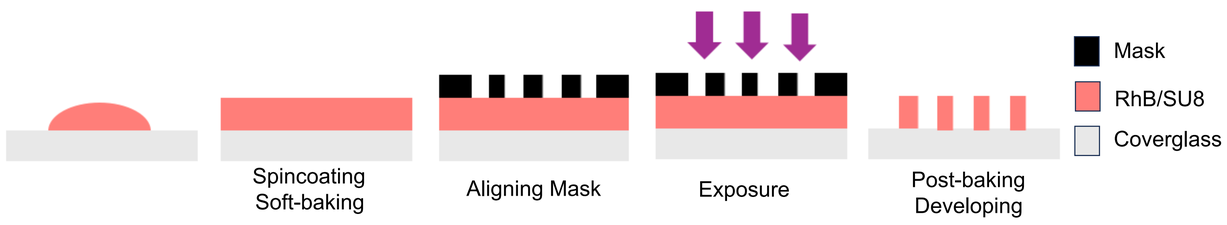
2) Determination of the concentration of fluorescent dye with SU8

Samples containing 0.01 mM, 0.1 mM, 1 mM, 10 mM, and 100 mM RhB/SU8 were prepared and examined.

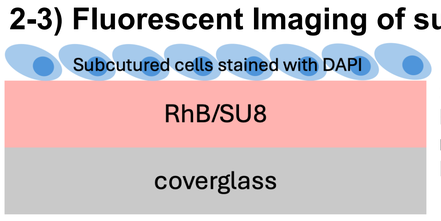
2-1) Spincoating : examine the uniform distribution of RhB and determine the presence of aggregation



2-2) Lithography : examine whether proper patterning can be done

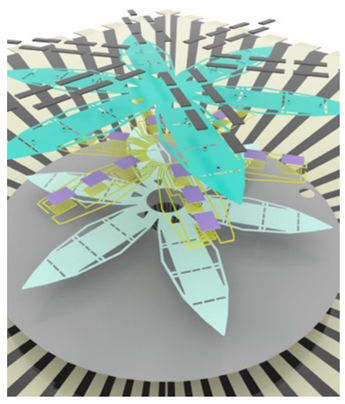


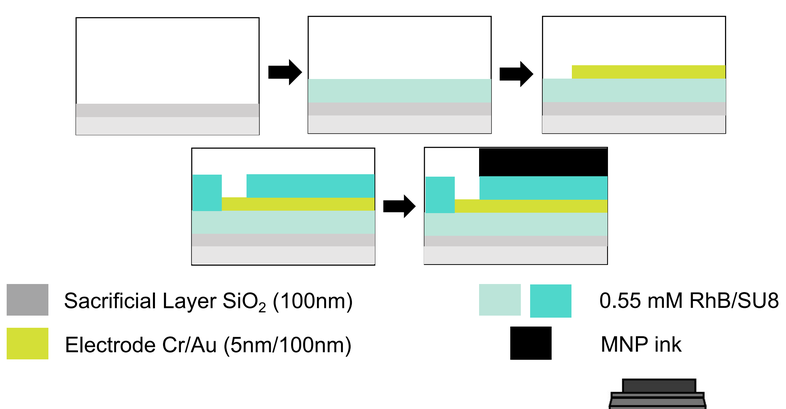
2-3) Fluorescent Imaging of subcultured cells on lithographed coverglass



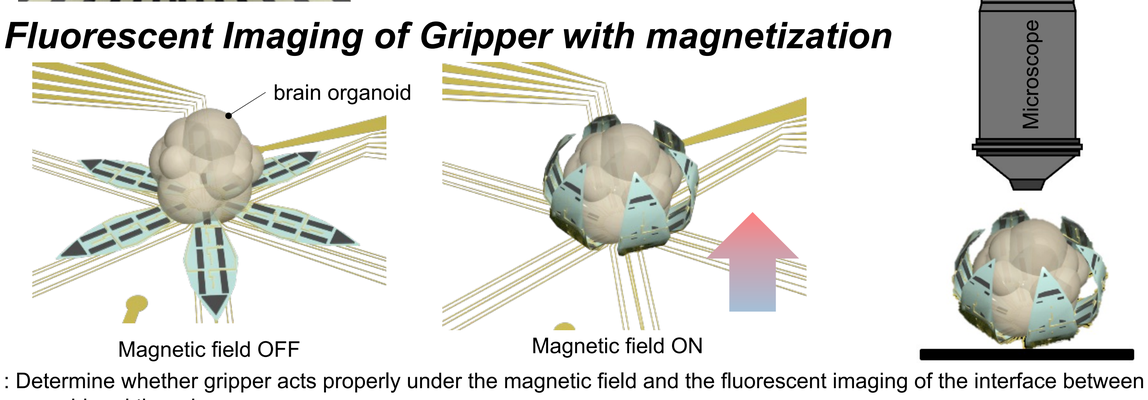
: Determine which concentration of RhB/DAPI shows the similar intensity Patterns were lithographed on the coverglass using each 0.01 mM, 0.1 mM, 1 mM RhB/SU8. Cells were subcultured on the coverglass and was stained using DAPI.

Lithography & Magnetic printing





Fluorescent Imaging of Gripper with magnetization



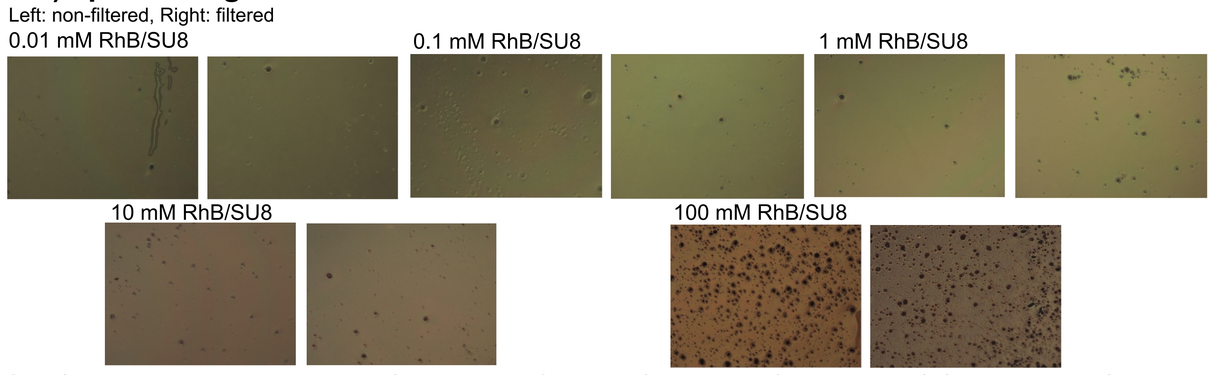
: Determine whether gripper acts properly under the magnetic field and the fluorescent imaging of the interface between the organoid and the gripper.

Results

Optimization of patternable polymer and dye

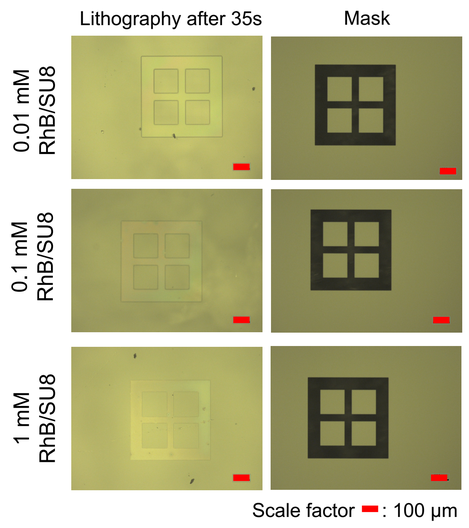
2-1) spincoating

Left: non-filtered, Right: filtered

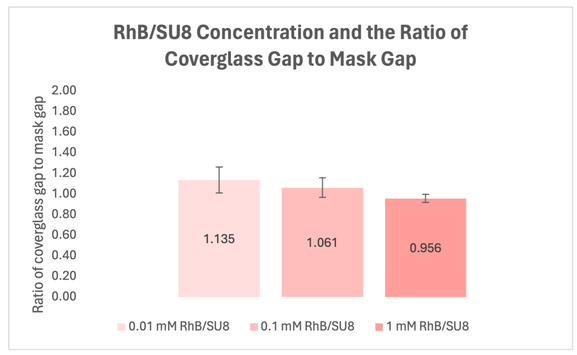


Samples 10mM and 100mM exhibited a large number of dye particles on the surface, which could lead to improper lithography. In contrast, samples 0.01mM, 0.1mM, and 1mM appeared to contain an adequate amount of dye particles, warranting further examination.

2-2) Lithography



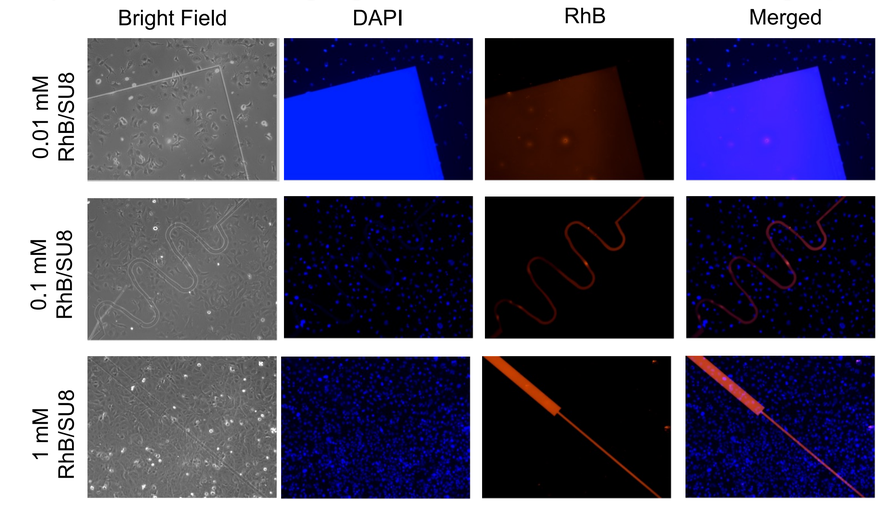
Scale factor ▬: 100 μm



y-axis: $ratio = \frac{coverglass\ gap}{mask\ gap}$

A ratio closer to 1 indicates that the lithography was performed well and that the lithography conditions were appropriate. The ratios for 0.01 mM RhB/SU8, 0.1 mM RhB/SU8, and 1 mM RhB/SU8 were all close to 1, confirming the suitability of these conditions.

2-3) Fluorescent imaging of subcultured cells on lithographed coverglass



Intensity of RhB is weak. Merged picture shows that only DAPI is detected.

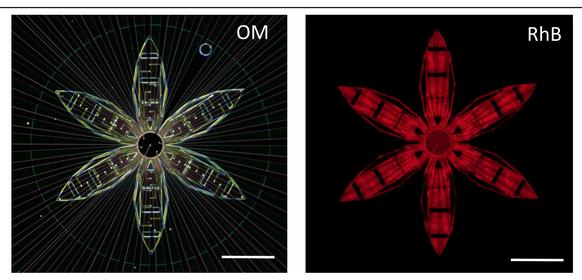
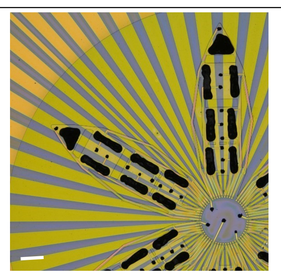
Merged picture shows intensity of DAPI is relatively stronger than that of RhB.

Merged picture shows that the intensity of RhB was relatively stronger than that of DAPI.

Upon comparing the fluorescence intensities of DAPI and Rhodamine B (RhB), it was determined that the appropriate concentration range for RhB/SU8 is between 0.1 mM and 1 mM. This range ensures that the fluorescence intensity of RhB/SU8 matches that of DAPI, allowing for optimal imaging and accurate visualization of the interface.

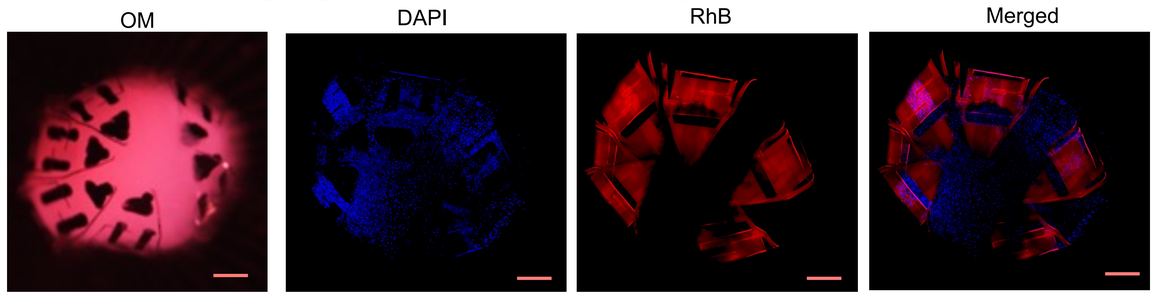
Accordingly, **<u>RhB/SU8 concentration was optimized to be 0.55 mM</u>**.

Lithography & Magnetic printing

OM image of gripper	Magnetic printing
	
Scale factor ▬: 1 mm	Scale factor ▬ : 200 μm

Fluorescent Imaging of Gripper with magnetization

Scale factor ▬ : 200 μm



The close interface between the gripper and the brain organoid was seen through the merged fluorescent image of both RhB and DAPI.

Conclusion

The primary goal of this experiment was to observe a stable interface between the gripper and the organoid. Various experiments were conducted to optimize the lithography conditions of the patternable polymer and dye, with a particular focus on the concentration of the fluorescent dye, Rhodamine B (RhB). Through the process of spin-coating, lithography, and fluorescent imaging using cells stained with DAPI, the RhB/SU8 concentration was optimized at 0.55 mM. Subsequent lithography and magnetic printing techniques were employed to fabricate the gripper, and fluorescent imaging confirmed the establishment of a stable interface between the organoid and the gripper.

Such advancements in organoid sensor technology are anticipated to significantly enhance our capacity to study complex cellular processes in a more representative and comprehensive manner, ultimately contributing to breakthroughs in biomedical research and therapeutic development.

Reference

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[2] Paul Le Floch, et al. “Stretchable Mesh Nanoelectronics for 3D Single-Cell Chronic Electrophysiology from Developing Brain Organoids.” Advanced Materials, Jan. 2022, https://doi.org/10.1002/adma.202106829

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