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# Self-healing Cell Tactile Sensor Fabricated Using Ultraflexible Printed Electrodes

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*Abstract*— **We used cells, which are the units that make up a living body, as building blocks to design a biomachine hybrid system and develop a tactile sensor that uses living cells as sensor receptors. We fabricated a novel cell tactile sensor with the electrodes formed using printed electronics technology. This sensor comprises elastic electrodes mounted on a soft material to acquire tactile information; similar to a conventional cell tactile sensor, it acquires signals through mechanical stimulation. Further, self-organization of cells can be induced, and logical processing such as selective responses to stimuli can be performed directly by the physical system, without any coding using programming languages. The proposed novel cell tactile sensor that uses printed electrodes is small enough to mount on robots. Interestingly, we confirmed the self-healing properties of the proposed sensor after cells were injured mechanically.**

## I. INTRODUCTION

Organisms have excellent abilities such as self-modification and self-healing, and they adapt to the environment by changing their physical structures [1]. Bio-machine hybrid systems have been attracting a lot of attention as a system that implements such excellent capabilities similar to living organisms on machines. In drive systems, various microrobots are driven by the beating of cardiomyocyte [2]–[6]. Telescopic actuators composed of skeletal myocytes [7]–[11] have also been developed. In this research, we focused on using cells, which are the units that make up a living body, as building blocks in a biomachine hybrid system and aimed to develop a tactile sensor that uses living cells as sensor receptors. Because the cell size is on the order of micrometers, a sensor built from cells would have a resolution sufficiently comparable with those of traditional tactile sensors [12].

Cells can induce responses to mechanical stimulations. Immediate mechanical stimulations lead to biochemical reactions [13]–[17]. On the other hand, long-term mechanical stimulations affect cell orientations and distributions [18]–[22]. In our previous research, we developed a cell tactile sensor that selectively responds to mechanical stimuli experienced by utilizing these unique properties of cells [23], [24]. We used cells cultured on a polydimethylsiloxane (PDMS) thin film as receptors; on applying mechanical stimulus through the thin film, the receptor cells can perceive tactile sensation. The thin film causes a mechanical change in the cell scaffold (i.e., PDMS thin film). Cells can be memorized through culturing by applying a training stimulus and can respond selectively to stimuli. This tactile sensor was used for measuring intracellular fluorescence due to calcium ion influx resulting from the reception of mechanical stimuli by the cells. Using this methodology for obtaining tactile information requires large measurement equipment. Thus, miniaturization becomes crucial when such equipment has to be mounted on robots (for installing on robotic fingers).

Therefore, considering these factors, in this research, we developed a novel method for acquiring tactile information using printed-electronics, which do not require a large detector (such as that used in a fluorescent microscope), and a small mountable cell tactile sensor. Upon receiving a stimulus, ions flow into the cell. Tactile information is obtained by measuring the extracellular potential change caused by the influx of ions at the cell-electrode adhesive interface. The measurement method using printed electrodes not only allows miniaturization but also eliminates the need to administer a fluorescent reagent to cells, and it can acquire signals as they are from cells. In the conventional tactile information acquisition method using fluorescence observations, calcium ions remain in the cell for a certain period after a stimulus is applied to the cell and intracellular ions rise (This deteriorates the time resolution of the sensor.). Because the tactile information acquisition method using electrodes involves measuring electrical changes outside the cell, it is not necessary to consider the residual calcium ions in the cell. Therefore, this sensor can function as a cell tactile sensor with improved responsiveness.

We developed a novel cell tactile sensor with electrodes fabricated using printed electronics technology. Printed electronics technology is used to form electronic circuits and devices through printing using conductive ink on material sheets. Compared to electrodes that have Au or Pt deposited on a glass plate used for measuring spikes in nerve cells, these electrodes can be printed more easily using a stretchable film or a soft material base. In this sensor, elastic electrodes are mounted on a soft material to acquire tactile information through mechanical stimulation, similar to a conventional cell tactile sensor. Self-organization of cells

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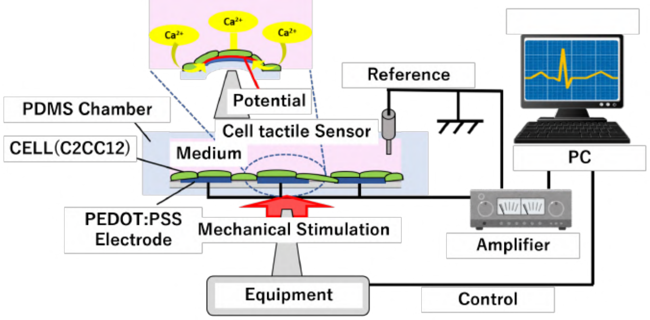

Fig. 1. Schematic diagram of Extracellular potential measurement.

can be induced, and logical processes such as selective responses to stimuli can be implemented directly on the physical system, without any coding using programming languages. The proposed novel cell tactile sensor that uses printed electrodes will be small enough to install on robots. Interestingly, we confirmed the proposed sensor has self-healing property after cells are injured mechanically.

## II. EXTRACELLULAR POTENTIAL MEASUREMENT METHOD USING PRINTED ELECTRONICS

In this study, to solve miniaturization the problem described in the previous section, tactile information obtained through extracellular potential measurements using electrodes fabricated via printed electronics technology was used as an interface to efficiently detect cell responsiveness without requiring a large detector. Fig. 1 shows a schematic of the method for acquiring tactile information by measuring the extracellular potential using electrodes. Tactile information is obtained by measuring the extracellular potential change caused by the influx of ions at the cell-electrode adhesive interface. Traditionally, this method has been used for spike measurements in nerve cells. A multipoint planar electrode (MEA), in which Au or Pt is deposited on a glass plate in a grid pattern, is commonly used [25]–[31]. We conducted a preliminary experiment with an MEA (MED 64 system, Alpha MED Scientific Inc. [32]). The extracellular voltage changes in the mouse skeletal myoblast C2C12 cells used in this sensor were measured. We found that C2C12 cells caused an extracellular voltage drop of -5 to -10 mV when receiving a mechanical stimulus. This result also indicates that it is easier to measure the voltage changes in C2C12 cells than nerve cells (extracellular voltage drop of nerve cells of approximately 100 $\mu$V).

Here, an important factor is that the mechanical stimulus to the cell is applied through the membrane to which the cell adheres. The applied stimulus deforms the membrane. Therefore, rigid culture dishes like MED 64 systems are not suitable for our purpose. We utilized electrodes fabricated using printed electronics technology. Printed electronics technology involves forming electronic circuits and devices through printing using conductive ink on a sheet-like object. The electronic circuits are printed easily on films with elasticity. The main advantage of this technology is that printing is performed on a soft material base. Therefore, using electrodes printed on a thin elastic film and culturing cells on them, mechanical stimulation is applied to the cells, and tactile information can be acquired from cells at the same time.

The proposed method can reduce the size of the cell tactile sensor compared to that used in the fluorescence observation method. We expected improved responsiveness from this sensor. Because measurements are performed at the adhesive interface between the cell and electrode, there is no need to consider residual calcium ions inside the cell. The calcium ions in the surrounding solution are immediately supplied, and the potential returns to a steady state. Thus, the solution becomes a buffer, and the responsiveness of the sensor will improve.

## III. DESIGN OF CELL TACTILE SENSOR

This section introduces a sensor interface that has a tactile information acquisition system based on extracellular potential measurements obtained using printed-electronics electrodes.

### *A. Printed Electronics on PDMS Thin Membrane*

Printed electronics have developed technology for producing conductive patterns, electrically passive elements [33], and even semiconductor elements [34] on a polymer substrate using printing technology. In this study, a mechanical stimulus is applied through the surfaces of the electrodes fabricated using printed electronics technology. This technology is useful when it is necessary to add cells and for self-organization of cells. Au and Ag are well-known materials for printed-electronics electrodes [35]–[37]. Electrodes suitable for the cell tactile sensor were selected and designed according to the following criteria:

1) The cells should adhere to the electrode.
2) The electrodes should be elastic to enable mechanical stimulation to be applied to the cells through the adhesive surface (electrode surface).
3) The electrodes should measure extracellular voltage changes occurring at the cell-electrode adhesive interface.

Poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) organic electrodes printed on polystyrene-polybutadiene-polystyrene (SBS) nanosheets [38] were used

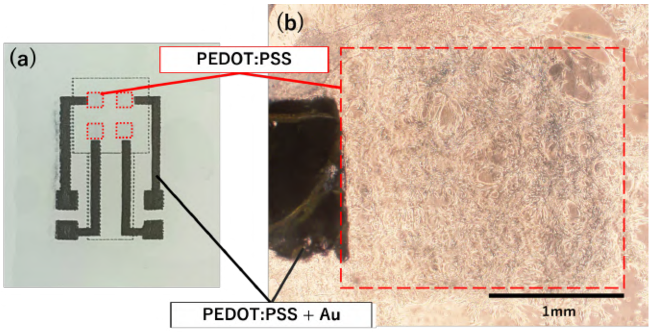

Fig. 2. PEDOT:PSS electrode shown in the image of a phase-contrast microscope.

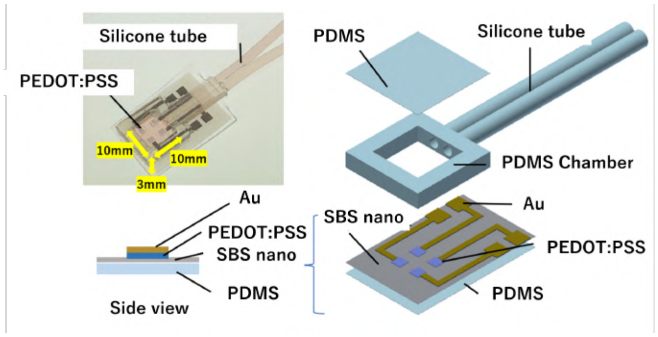

Fig. 3. Outline of cell tactile sensor

as the electrodes for this sensor because they satisfied the above design requirements. The PEDOT:PSS electrodes printed on SBS nanosheets used in this sensor are shown in Fig. 2(a). These electrodes are transparent and stretchable organic electrodes. As these electrodes are organic, these are moderate for interfacing the electrode to the cells, thus meeting requirement 1). Moreover, owing to their transparency, they help observe the state of cell adhesion with a phase-contrast microscope. The phase-contrast image of a cell (C2C12) adhered to the PEDOT:PSS electrode is shown in Fig. 2 (b). Furthermore, the electrodes meet requirement 2) because both the PEDOT:PSS electrodes and SBS nanosheets have elasticity, and the Young’ s moduli of ultrathin films formed using SBS (thickness 690 nm) and PEDOT:PSS (thickness 40 - 120 nm) are 4.9 MPa and 1.0 GPa, respectively [39], [40]. Therefore, a mechanical stimulus can be applied to the cells through the adhesive surface (electrode surface). Thus, tactile information from cells can be obtained. Regarding requirement 3), the impedance between the electrode and conductor is important to measure the extracellular voltage generated at the cell-electrode adhesive interface. By printing Au after PEDOT:PSS, the impedance becomes lower, allowing for the stable measurement. Also, since cells do not adhere to Au, cells adhere only to the electrode part of PEDOT:PSS, which is the measurement point, and Au is used for the conductor part. It functions as a mask and is designed so that cells do not adhere.

### B. *Cell Tactile Sensor Device*

The cell tactile sensor was created using the electrodes described above. Fig. 3 shows the picture and structural diagram of the designed cell tactile sensor. The cells (*i.e.*, the receptors of the tactile sensor) are cultured on SBS sheets. The cells can be cultured in the space surrounded by the PDMS chamber and membrane. Additionally, the mounted silicone tube facilitates cell seeding and exchange of the culture medium. As the printed PEDOT:PSS electrode is flexible yet ultrathin, it cannot withstand the mechanical stimulus applied from under the sheet. Therefore, a PDMS thin film is stuck under the SBS sheet to form a two-layered structure.

## IV. TACTILE INFORMATION FROM CELLS WITH PRINTED ELECTRONICS

We used the cell tactile sensor described in the previous section to acquire tactile information from cells. We conducted the experiment after confirming that the cells had adhered to the electrode. Specific mechanical stimuli were applied to the cells from under the thin-film electrode to which the cells were adhered using a mechanical stimulus application device. Then, the changes in the extracellular potential caused by the influx of ions that occurred during the measurement were observed. We determined the type of information that could be obtained as tactile information from the measured signal resulting from the extracellular potential changes.

Because the extracellular potential change provides a small signal, it was measured through an amplifier and a filter circuit. In the following subsections, the preparation of the cells as receptors for the sensor used in our experiment, designed mechanical stimulus application device, and amplifier and filter circuit are explained. Then, we present the experimental results.

### A. *Cell Preparation*

Mouse skeletal muscle myoblast C2C12 cells were used as the receptors of this cell tactile sensor. For seeding the cells into the sensor, the cell tactile sensor (see Fig. 3) was sterilized using ultraviolet light for 2 min. We seeded the C2C12 cells using Dulbecco’s Modified Eagle’s medium (D5796, containing 10% fetal calf serum and 1% penicillin streptomycin). After seeding, we placed the cells in a $CO_2$ incubator at 37°C and 5% $CO_2$ concentration for 6 h until the cells adhered to the electrodes. Figure 4 shows the C2C12 cells adhered to the PEDOT:PSS electrode.

### B. *Design of Mechanical Stimulus Application Device*

A mechanical stimulus application device was designed to apply mechanical stimuli to the cell tactile sensor quantitatively and measure the responsiveness (see Fig. 5). The stimulus application part made of polylactic acid (PLA)

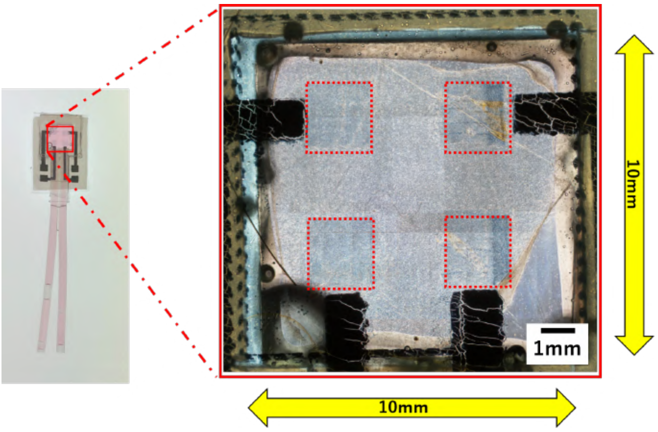

Fig. 4. Adhesion of cells to PEDOT:PSS electrode. Red rectangles indicate transparent PEDOT:PSS electrode. As the figure explains, Cells adhere to the electrode.

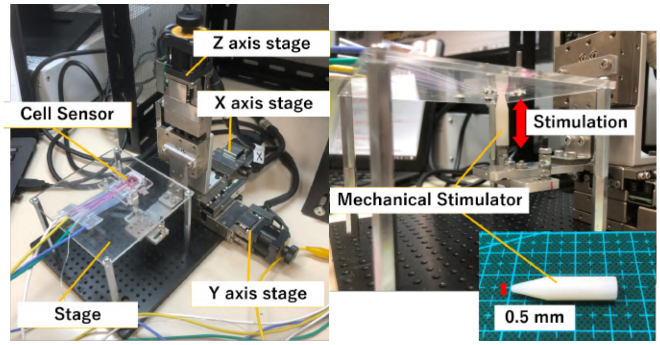

Fig. 5. Mechanical stimulus application device

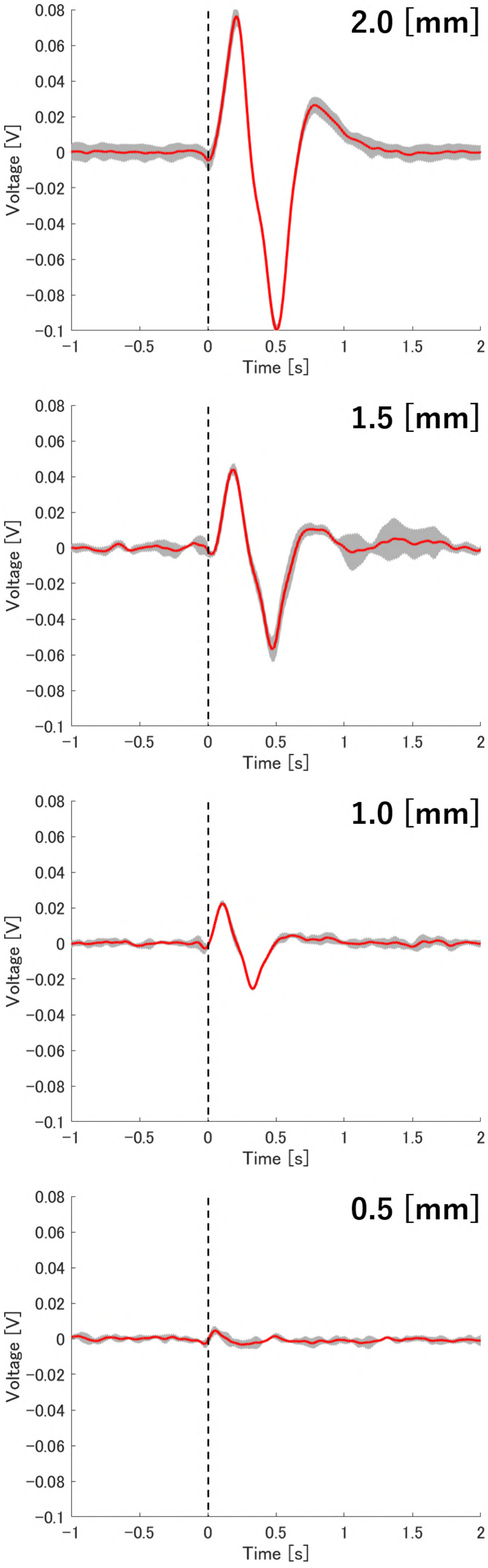

Fig. 6. Average and standard error of each stimulus application for the data of 6 stimuli applied during 1-minute measurement. The stimulus was applied at 0 s.

resin is pushed up from under the membrane to apply mechanical stimuli to the cells. At this time, an arbitrary thrust is provided at an arbitrary stimulation point on the sensor electrode surface. This is designed to be driven in the horizontal (X- and Y-axes) and vertical (Z-axis) directions. The equipment is actuated by a Suruga Seiki motorized stage with a PC and a controller (DS102, SURUGA SEIKI), and it is possible applying stimuli at the micron level.

### C. *Experiments to Acquire Tactile Information by Measuring Extracellular Potential Changes*

Using the cell tactile sensors and devices introduced above, we conducted experiments to acquire tactile information by measuring extracellular potential changes. Specifically, we investigated what type of data could be obtained as tactile information from the measured extracellular potential change signal. The differences in the measured signals were determined by changing the intensity of the mechanical stimulus applied to the cell tactile sensor. Mechanical stimulus was applied to the sensor using the mechanical stimulus application device, and the signal obtained from the electrode was sent to an analog amplifier filter circuit and then amplified to remove the noise. The output signal from the analog amplifier filter circuit was analyzed using LabVIEW.

### D. *Comparison of the Extracellular Potential Changes*

We observed how the extracellular potential changed when the intensity of the mechanical stimulus applied to the cell tactile sensor changed. The electrode was placed at the center of the electrode membrane of the cell tactile sensor. The mechanical stimulus application device applied thrust from under the membrane. The thrusting speed was set to 20 mm/s. At that time, the level of thrusting was varied. The thrusting lengths were 2.0, 1.5, 1.0, and 0.5 mm. The measurements were obtained once every 10 s. The measurements were performed for 1 min. Fig. 6 shows the measurement results. The graph shows the average values and standard errors of the measured values, plotted using the measurement data obtained by applying the mechanical stimulus six times per minute. In addition, a 10-Hz digital low-pass filter was used during MATLAB processing. According to Fig. 6, the obtained signals for thrusting lengths of 1.0 - 2.0 mm were large compared to noise. This result was attributed to the amplitude of the extracellular potential change caused by applying the mechanical stimulus at 10-s intervals with the mechanical stimulus application device. Fig. 6 shows the average and standard error of the measurements obtained by applying the stimuli six times during 1-min measurements. The stimulus was applied at 0 s in that graph. The red line is the average value and the gray area is the standard error. When the thrusting length was 0.5 mm, the amplitude of the mechanical stimulus was small, but the waveform corresponding to the mechanical stimulus could be observed. The amplitude of the mechanical stimulation was successfully measured. The measured value was found to increase in proportion to the thrusting lengths.

## V. SELF-ORGANIZATION ABILITY

Cells have self-organization abilities and can change their structures, distributions, and orientations in response to long-term mechanical stimulation. In our previous study [23], we developed a cell tactile sensor that memorizes and responds selectively to mechanical stimuli. To achieve such stimulus selectivity in this study, we developed a cell tactile sensor that exhibits self-organization through mechanical stimulation. We verified whether tactile information changes following self-organization of the cells in the proposed cell tactile sensor.

### A. *Experimental Setup*

When a stretching stimulus is applied to the adherent scaffold, the cell is oriented perpendicular to the direction of extension. Based on this property, cells cultured on an elastic membrane can be used. Then, we can control the cell distribution and orientation through mechanical stimuli. The stimulus selectivity of the cell tactile sensor is largely related to the change in orientation when a specific stimulus trains cells. In our experiments, we induced self-organization of the cells in the proposed cell tactile sensor and investigated whether the responsiveness of the sensor changed.

Specifically, a mechanical stimulus (poking stimulus) was applied to the center of the electrode film in the sensor to orient the cells in a circular shape based on our previous study. First, C2C12 cells were seeded on a PDMS thin film using the same PDMS chamber as in the present sensor. We confirmed that cyclically oriented cell structure was formed on applying a 2-mm poking stimulus at 0.5 Hz periodically for 3 h. Cells were oriented in a cyclic pattern on the PEDOT:PSS electrode membrane and responsiveness comparison experiments were performed.

### B. *Experimental Result*

Mechanical stimulation was applied to the electrode membrane of this sensor to change the orientation of the cells. The orientation is shown in Fig. 7. The response of the tactile sensor before and after orientation was compared, and the results are shown in Fig. 8. The response data were obtained by applying a pushing stimulus of 2 mm to the center of the electrode film every 10 s with measurements recorded for 1 min. Then, the data from four electrodes were combined, as shown in Fig. 8. The stimulus was applied at 0 s in that graph. As in the graph, the larger response was observed in the condition of ”after orientation (blue line)” than the condition of ”before orientation (red line)”. As explained earlier, mechanical stimulation of the cells affects calcium inflow into the cells. Therefore, this could mean a significant increased in calcium inflow occurred after the cell orientation changed. However, more detailed experiments should be conducted to clarify this phenomenon.

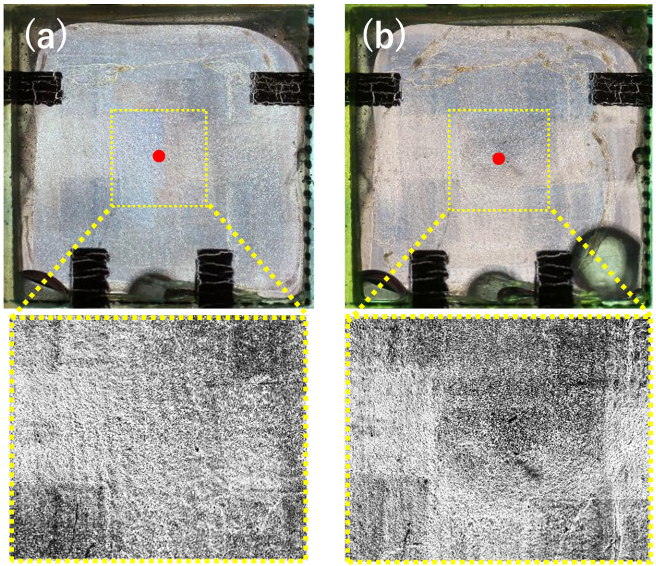

Fig. 7. Orientation of cells(a)Before orientation (b)After orientation. Red points are the stimulation points.

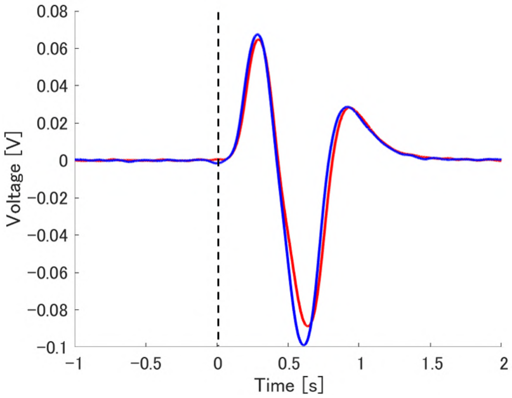

Fig. 8. Data from 4 electrodes were integrated. The voltage of the minus side after orientation(blue) was more significant than the data before orientation(red). The stimulus was applied at 0 s.

## VI. SELF-HEALING CELL TACTILE SENSOR

In general, tactile sensors use engineering components such as piezoelectric elements in their receptors. Thus, if a strong force is applied, the receptors can break. Consequently, the sensors must be replaced. However, in this study, because living cells were used, we expected that the self-healing ability of these living cells could repair the damage

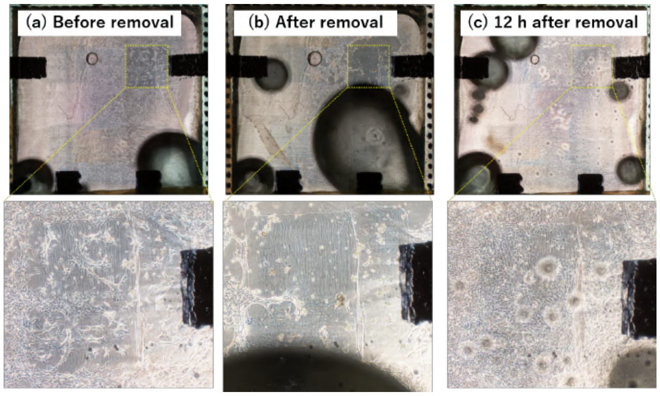

Fig. 9. Cells on the electrode reproduce by cell division at the detached part and adhere to the electrode again only by leaving it in the CO2 incubator for 12 hours.

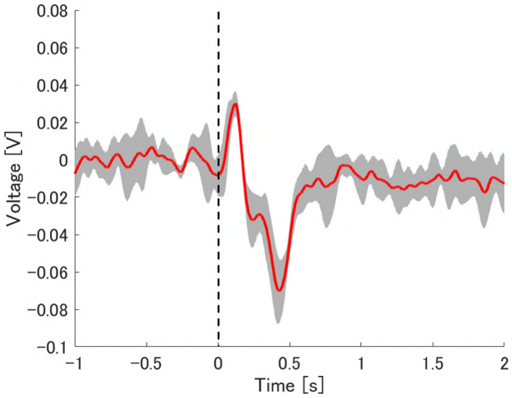

Fig. 10. Receptor responds using the revived cell tactile sensor. The stimulus was applied at 0 s.

caused on applying a force. Here, a simple experimental setup was employed to investigate the self-healing abilities of the cell tactile sensor. The experimental process is explained below.

1) The cells were intentionally removed from the electrodes.
2) The sensor was placed in the $CO_2$ incubator overnight and examined using a phase-contrast microscope to determine whether the cells returned to their original positions (whether the receptor is restored).
3) A mechanical stimulus was applied to the center of the electrode film to investigate whether it could respond again.

The results of the experiment are shown in Fig. 9. After removal, the cells on the electrode multiplied through cell division at the detached part and adhered to the electrode again only after leaving it in the $CO_2$ incubator for 12 h. Fig. 10 shows the receptor response when using the revived cell tactile sensor. The data were recorded at the center of the electrode film. The data at the upper right electrode were measured when a thrust stimulus of 2 mm was applied at 10-s intervals for 1 min. The stimulus was applied at 0 s in that graph, and the average of the measured values and the standard error were determined. An analog amplifier filter (amplification factor 200 times, a 10-Hz low-pass filter, and a 1.5-Hz high-pass filter (Digital filter Low-pass filter 10 Hz) were used for processing. The figure shows a large amplitude resulting from mechanical stimulation. Therefore, tactile information can be acquired using this cell tactile sensor. Thus, we can expect that the proposed cell tactile sensor may exhibit self-healing properties even if it is crushed or broken during use.

## VII. CONCLUSIONS

In this study, we developed a tactile sensor that can adapt to the environment using living cells as receptors. In our previous research [23], we used the self-organizing ability of the cells experiencing mechanical stimulation. We developed a tactile sensor that responds specifically to mechanical stimulus. The main finding of this research is that the cells respond to mechanical stimuli applied from below the membrane after culturing the cells as receptors on a soft and stretchable PDMS thin film. In this study, tactile information was acquired by measuring changes in the extracellular potential using printed electronics technology. By developing this cell tactile sensor, we succeeded in utilizing the self-organizing capabilities of cells. Investigation of the responsiveness showed that the signal that could be measured increased according to the magnitude of the force applied. Therefore, the proposed cell tactile sensor can recognize the magnitude of the applied force.

Although this study developed a novel tactile sensor that uses living cells as receptors, there are still many issues to be investigated(*e.g.*, an amplifier makes the whole system larger.). There are experiments that should be conducted to use the cell tactile sensor in an engineering way. For example, force measurement range by the sensor, repeatability of the sensor, accuracy, sensitivity, resolution, effect of cell alignment on sensing accuracy, and so on, are important. One of the problems to be solved is disconnection of the wiring. The sensor is mainly composed of soft and elastic materials such as PDMS. The Au wire used as the mask to prevent the cells from adhering became more susceptible to disconnection owing to the differences in elastic modulus and strain between Au and PDMS. This problem could be solved if the conductive ink used for preparing electrodes is replaced with a less conductive one or a printing pattern is developed to prevent disconnection. We hope that if these problems can be solved and the responsiveness of the tactile sensor can be further improved, a novel cell tactile sensor with growth potential not found in engineering sensors till date will be realized.

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