# Fabrication and functional analysis of bio-actuator for soft robot

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**Abstract:** Our goal is development of a bio-actuator which is made of extracellular matrices and lower cell density skeletal muscle cells. In order to increase a contractile force of the bio-actuator for soft robot, it is necessary to find a composition of extracellular matrices which can affect the contractile force. In this study, we fabricated bio-actuators by changing the components of extracellular matrices and performed function analysis by electrical stimulation. These data indicated that the bio-actuator whose ingredients were matrigel and collagen performed the highest contractile force in this study.

Keywords: soft robot, artificial muscle, bio-actuator

# **1. INTRODUCTION**

Soft robots have been developed to give flexibility to robots. One of the soft robots is a robot with a skeletal muscle bio-actuator as a driving source. Bio-actuators use three dimensional tissues consisting of extracellular matrices and myoblasts (Fig.1). These bio-materials perform as a scaffold for cell growth and as a driving source, respectively. Since we use cells, this actuator is light weight, flexible, highly energy efficient and also expected to have the ability to adapt to the environment. However, the conventional bio-actuator [1,2] has a high cell density. If engineers increase the size for robot application, it will be difficult to secure cells as a material, and the cost will increase. Therefore, the purpose of this study is to develop a bio-actuator with a relatively lower cell density than conventional ones. Since it is known that the contractile force changes depending on the composition of the extracellular matrices from the conventional findings [3], in this report, we fabricated a lower cell density bio-actuator by changing the extracellular matrices and investigated its function.



Fig. 1 Conceptual diagram of Bio-actuator

## 2. EXPERIMENTAL METHOD

To develop lower cell density actuators, we fabricated them by changing the composition of the extracellular matrices and measure their contractile force by electric stimulation. For the composition of the extracellular matrices, we tried three combinations as shown in Table 1 below. As extracellular matrices in this experiment, we used Matrigel (356231, Corning, New York, the U.S.),

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Collagen (Collagen Gel Culturing Kit, Nitta Gelatin Inc., Japan), and Fibrinogen (F3879, Sigma Aldrich, Missouri, the U.S.). In the table, "+" means included, and "-" means not included. The experimental schedule is shown in Fig.2. We fabricated the bio-actuator with myoblasts and matrices so that the cell density becomes constant. The bio-actuators were fixed by pins for cell alignment [4]. Then, two or three days later, the growth medium was switched to the differentiation medium, and the differentiation medium was replaced on day 6. Growth medium promotes cell propagation and differentiation medium promotes cell differentiation so that myoblasts can grow up to be myotubes. Since the bio-actuator made of myoblasts contract by electrical stimulation, we measured this contractile force by electrical stimulation on day 8. In this case, we varied the applied voltage and application time. For electrical stimulation, the experimental setup is shown in Fig.3. We removed one of the two pins from the bio-actuator, and inserted a micro-force sensor (AE801, Kronex, California, the U.S.) [5] into the hole made by the inserted pin. Then we attached electrodes to the dishe sides on both ends of the bio-actuator and made a potential field.

 Table 1 Composition of extracellular matrices

 (+ : Included, - : Not included)

Condition	Matrigel	Collagen	Fibrinogen
i) C	-	+	-
ii) MF	+	-	+
iii) MC	+	+	-



Fig. 2 Experiment schedule



#### 3. RESULT

To evaluate the contractile force of each composition, we calculated the contractile force rate. We defined it as each force divided by the force of the condition i) C since the force of this condition i) C was the smallest contractile force. We prepared three samples of each composition, measured the force five times in each sample, and calculated the force rate. Fig.4 shows the comparison of mean contractile force rates in voltage, and Fig.5 shows the mean rates in application time. The alphabets on the figures show the statistical analysis results by the Kruskal Wallis test (p < 0.05). If the alphabets are different, it means that there is a significant difference. In Fig.4, though there was the significant difference in contractile force rate when the voltage was 2V, it was almost the same force rate. On the other hand, the significant difference appeared and there was the big gap between i) C and the others when the voltage was 30V. Especially, the composition of condition iii) MC performed the maximum contractile force rate. In Fig.5, there was the significant difference in contractile force rate when the voltage was 2V. However, no shrinkage was seen when the application time was 10 ms. On the other hand, a difference in the contractile force rates was seen and there was the big gap between i) C and the others when the application time was 100 ms. Especially, the composition of condition iii) MC performed the maximum contractile force rate as well. Therefore, we concluded that the composition of iii) MC, which contained matrigel and collagen, showed the maximum contractile force rate.



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Fig. 5 Comparison of contractile force rate in application time

### 4. CONCLUSION

In this study, we fabricated a bio-actuator by changing the composition of extracellular matrices, and measured contractile force as a functional analysis to develop a bioactuator with lower cell density. Results show that condition iii) MC including matrigel and collagen performed the maximum contractile force rate in both the applied voltage and application time.

In the future, we will fabricate a bio-actuator with a mixed composition of the three extracellular matrices (collagen, matrigel, and fibrinogen), and examine the conditions to increase the contractile force. We also plan to create a soft robot with the bio-actuator constructed in this research.

### 5. ACKNOWLEDGEMENT

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Fig. 4 Comparison of contractile force rate in voltage