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In situ monitoring of extracellular matrix based on chondrocytes behavior using biologically-coupled field effect transistor

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Abstract

In this study, we electrically measured the chondrocytes production behavior of extracellular matrix (ECM) using a biologically-coupled field effect transistor (bio-FET) sensor in a non-invasive, label-free and real-time manner. As a result of that, the increase of ECM membrane around chondrocytes was detected as the change of capacitance on the gate of the bio-FET, and confirmed by analyzing the quantity of sulfated glycosaminoglycan (sGAG) in the ECM. Thus, we showed the possibility of non-invasive and real-time method to evaluate cartilage cell for transplantation using the bio-FET.

1. Introduction

In the field of orthopaedics, there are a number of patients suffering from arthrosis. At present the medical techniques to transplant cultured chondrocytes have been developed and used in clinical pathology. Mizuno *et al.* reported that the biosynthesis of ECM was stimulated by a hydrostatic pressure loading, and the culture system of chondrocyte with the hydrostatic pressure loading has been studied for clinical trial [1]. However, the mechanism of synthesis of ECM by cultured chondrocytes has not been known well, so understanding and controlling the mechanism of differentiation are very important to improve the safety and reliability of transplantation of cultured chondrocytes. On the other hand, we have been developing the biologically-coupled field effect transistor (bio-FET) as one of the biosensing technologies. The bio-FET enables to detect various biological phenomena such as DNA molecular recognition events, antigen-antibody reactions and cellular activities, based on the good abilities of real-time, non-invasive and label-free detection [2,3], In this study, we have proposed the chondrocyte-based FET in order to evaluate the ECM production behavior of chondrocytes in a non-invasive and real-time manner.

2. Experimental section

2.1 Electrical monitoring of chondrocytes

The n-channel depletion type ion sensitive FET(IS-FET) was used as the monitoring device. The ratio of W/L was 10 μm/340 μm and the gate insulator were composed of SiO2, Si3N4 and Ta2O5. The bovine chondrocytes (isolated from femoral patellar groove, passage 2~3) and normal human chondrocytes (purchased from Lonza Japan, isolated from knee, passage 4) were transferred on the gate sensor surface of FET devices. After the cultivation overnight, both chondrocytes were confluent on gate area. The change of gate surface potential was continuously monitored for 2 weeks using the chondrocyte-based FET with or without ascorbic acid (5 μg/ml), although the culture medium was exchanged every 3 days (ascorbic acid was added after first medium change). After the measurement, the morphological properties of chondrocytes were observed using upright microscope.

2.2 Quantitative assay of sulfated glycosaminoglycan (sGAG)

We measured the quantity of sGAG by Alcian Blue dyeing. The cells and medium used in this experiment were same as electrical measurement. The bovine and normal Human chondrocytes were transferred to 4 well dish, respectively. The chondrocytes on 2 wells were cultivated with ascorbic acid, and other 2 wells were cultivated without ascorbic acid. The quantity of sGAG was measured at the 2nd, 5th, 8th, and 11th days after the start of cultivation.

3. Result and discussion

Fig. 1 shows the result of electrical measurement. In the case of bovine chondrocyte without ascorbic acid (red line of (b)), cyclic potential change was monitored after every medium change. We think the medium change activated the chondrocytes' respiration activity resulting in the increase of hydrogen ion with positive charge. Moreover, in the case of bovine chondrocytes with ascorbic acid (blue line of (a)), the decline of surface potential was clearly monitored. This signal was the most distinctive in Fig.1. On the other hand, in the case of normal human chondrocytes with or without ascorbic acid (green and purple lines of (c) and (d)), the surface potential did not change so much.

Fig. 2 shows the result of the measurement of sGAG quantitative assay. In the case of bovine chondrocytes, the

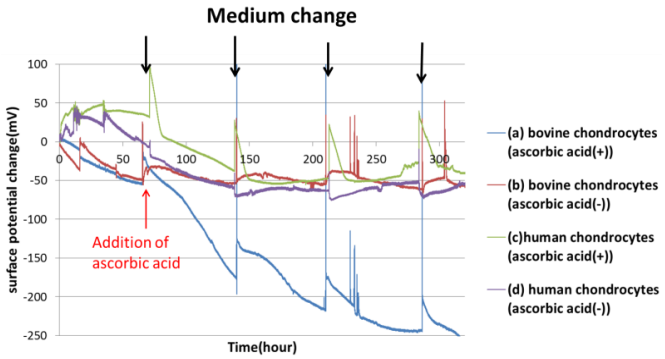


Fig. 1 Surface potential changes during the cultivation of chondrocytes with the hydrostatic pressure loading.

quantity of sGAG drastically increased from 5 days to 8 days for them with ascorbic acid although the quantity of sGAG did not change so much for them without ascorbic acid. On the other hand, in the case of normal human chondrocytes, the quantity of sGAG was finally very little for

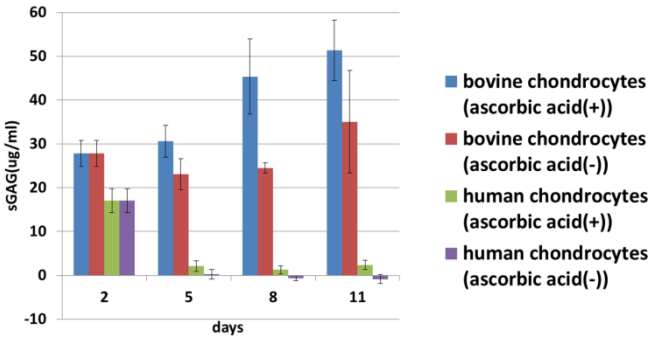


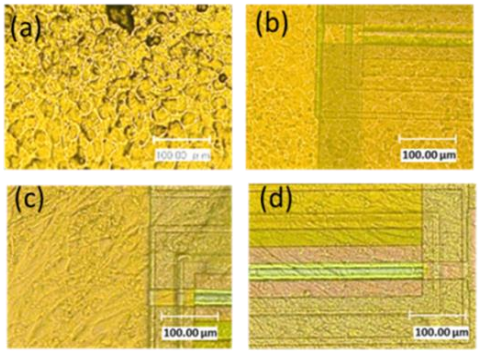
Fig.2 The quantity of sGAG production from chondrocytes with or without ascorbic acid.

them regardless of with or without ascorbic acid.

Fig. 3 shows the morphological properties of chondrocytes on FET sensor for 2 weeks after the cultivation. The bovine chondrocytes without ascorbic acid showed polygonal cellular shape, and the human chondrocytes showed the fibrous cellular shape regardless of with or without ascorbic acid. Because of their phenotypical difference, it was considered that the human chondrocytes were likely to be dedifferentiated and lost the ability to synthesize the ECM. It was thought that the result of electrical monitoring and sGAG quantitative assay reflected chondrocytes' dedifferentiation.

On the other hand, the bovine chondrocytes with ascorbic acid showed the three-dimensional shape compared with other samples. It was likely that the membrane of chondrocytes covered with matured ECM worked as insulator and induced the total capacitance change (Fig. 4).

In this experiment, the surface potential was measured by monitoring gate-source voltage change at the constant drain current, thus this capacitance change would induce the drastic surface potential change.



(a) bovine chondrocytes(ascorbic acid(＋))
(b) bovine chondrocytes(ascorbic acid(－))
(c) human chondrocytes(ascorbic acid(＋))
(d) human chondrocytes(ascorbic acid(－))

Fig. 3 Microscopic images of chondrocytes on sensing area of IS-FET.

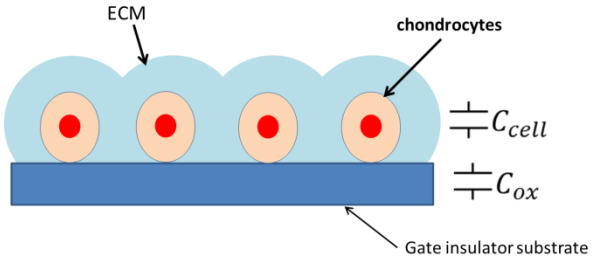


Fig. 4 Image of capacitance change by ECM membrane.

3. Conclusions

In this study, we tried to monitor the ECM production behavior of chondrocytes by using the bio-FET sensor. As a result, the surface potential change was clearly monitored in the case of bovine chondrocytes stimulated by ascorbic acid. Moreover, the quantity of sGAG drastically increased and the chondrocytes on the FET sensor showed three-dimensional shape in the same condition. We think the synthesized ECM around chondrocytes as well as stimulated chondrocytes caused the capacitance change resulting in the surface potential change. The electrical measurement using bio-FET sensor was likely to monitor the process of the ECM synthesis.

References

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