EFFECT OF PULSE ELECTRIC FIELD STIMULATION ON CHONDROCYTES

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1. Background

Articular cartilage covers the articulating bone ends of diarthrodial joint, and it has important functions of load bearing, impact absorption and low friction. In cases of severe joint diseases such as osteoarthritis, arthroplasty is applied for the patients. However, a lifetime of joint prosthesis is about 10 years due to loosening of the implant and wear of ultra high molecular weight polyethylene tibial insert as major problems. Therefore the transplantation of cultured cartilage has been studied recently. Since cultured cartilage does not have enough stiffness to bear body weight, it is necessary for the cultured cartilage to have the same mechanical property as natural cartilage in a short period.

It was reported that mechanical stimulations such as compression and hydrostatic pressure that simulated physiological environment accelerated activity of chondrocytes [1]. However mechanical stimulations may cause contamination, because loading devices touch the tissue directly to apply the stimulation.

Frank *et al*. reported that a streaming potential occured in cartilage when articular cartilage was compressed by an applied load [2]. Electric stimulation in cartilage tissue is caused by the everyday activities such as walking, it is considered that chondrocytes are received the electric stimulation in addition to the mechanical stimulation. These stimulations may accelerate activity of chondrocytes.

The purpose of this study is to investigate the effect of values of electric field stimulation on the activity of chondrocytes in the culture process.

2. Methods

2-1. Electric field stimulation system

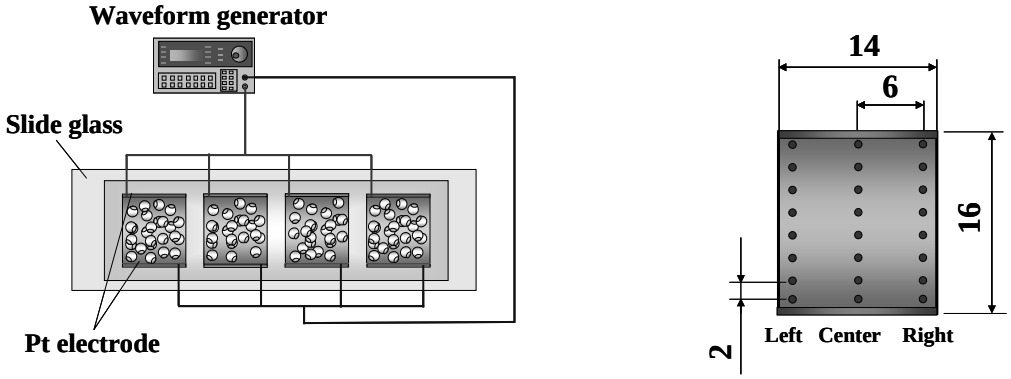
Fig. 1(a) shows the electric field stimulation system. This system consisted with the custom-made culture chamber and a function generator (WF 1984, NF Corporation). The custom-made culture chamber has a slide glass on the bottom and parallel platinum electrodes in each well.

Since it is thought that body weight is applied to a knee joint like impact, the electric field stimulation was applied by using a pulse wave. Electric field values were 5, 10 ,25 and 50 mV/mm. Pulse widths were 2, 15 and 25 ms. Frequency of a pulse wave was 1 Hz according to walking cycle.

2-2. Electric field value measurement

The electric field was confirmed for each well of the custom-made culture chambers. Electric potential was measured at 24 points in each well as shown in Fig. 1(b). Electric potential was

measured with an oscilloscope (WaveJet 300A, Iwatsu Electric Co. LTD) and a probe made by a stainless steel wire. The electric field value was calculated from potential gradient.



(a) Electric field stimulation system (b) Electric potential measureing points

Figure 1 The custom-made culture chamber.

2-3. Specimens

Chondrocytes were isolated by enzymatic digestion of collagenase from articular cartilage of porcine knee joint. The isolated chondrocytes were seeded in Dulbecco's modified Eagle's medium supplemented with 10 % FBS, antibiotic and ascorbic acid at a concentration of 2.0×10^6 cells/ml. The cellular suspension of 1.1ml (1.0×10^4 cells/mm^2) were seeded into each well. The custom-made culture chambers were in 37 ˚C, 5 % CO_2 incubator. The chondrocytes were cultured for 48 hours with electric field stimulation as the stimulation group and the chondrocytes were cultured without electric field stimulation as the control group.

2-4. Microscopic observation

Morphology of chondrocytes were observed using a phase contrast microscope (IX71, OLYMPUS) after 0, 24 and 48 hours cultivation.

2-5. Cell activity evaluation

Amount of the chondroitin sulfate that was main component of the proteoglycan was measured as the index of the cell activity. The amount of chondroitin sulfate in culture medium was measured by DMMB method with the spectrometer after cultivation [3].

3. Results

3-1. Electric field value measurement

Fig. 2 shows the result of electric field value measurement at 50 mV/mm electric field. The X-axis shows the distance from cathode electrode. The Y-axis shows electric potential. The electric field values of left, center and right of the well were 51.5, 52.6 and 50.3 mV/mm respectively. Since electric potentials of the left, the center and the right of a well were almost the same, the electric field in the custom-made culture chamber was uniform and perpendicular to the electrode.

3-2. Microscopic observation

Fig. 3 shows microscopic photographs of the center of the well for the control group and the stimulation group (electric field value : 50 mV/mm, pulse width : 25 ms) after 0, 24 and 48 hours cultivation. The extracellular matrix synthesized around the chondrocytes is observed with cultivation. The electric field stimulation do not affect the cell morphology and dead cells by the pulse electric field stimulations are not observed. Dead cells were not obserbed near the electrode.

3-3. Effect of pulse electric field stimulation on chondrocyte

Fig. 4 shows results of the amount of chondroitin sulfate measurement. Chondroitin sulfate is normalized by mean value of the control group for each test. When the pulse width is 25 ms, cell activity decreases with increasing electric field value. In the case of 15 ms pulse width, cell activity increase at 5 mV/mm electric field, whereas cell activity decrease when electric field value is over 25 mV/mm. When the pulse width is 2 ms, cell activity increase at 25 mV/mm electric field.

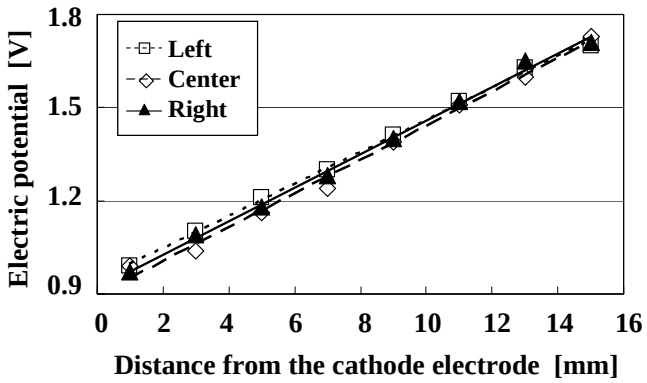


Figure 2 Result of electric field value measurement (electric field value : 50 mV/mm).

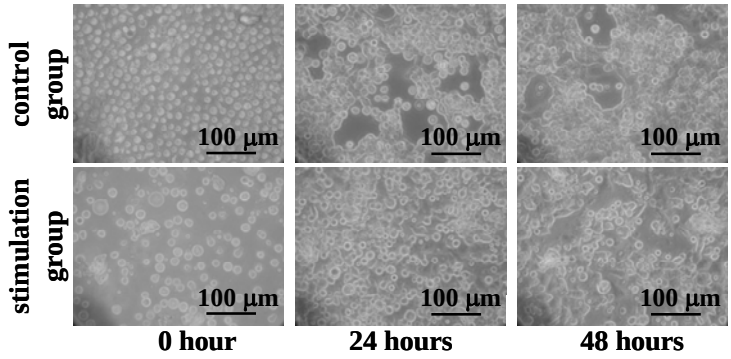


Figure 3 Microscopic photographs of chondrocytes after 0, 24 and 48 hours.
(Stimulation group : electric fied value of 50 mV/mm, pulse width of 25 ms)

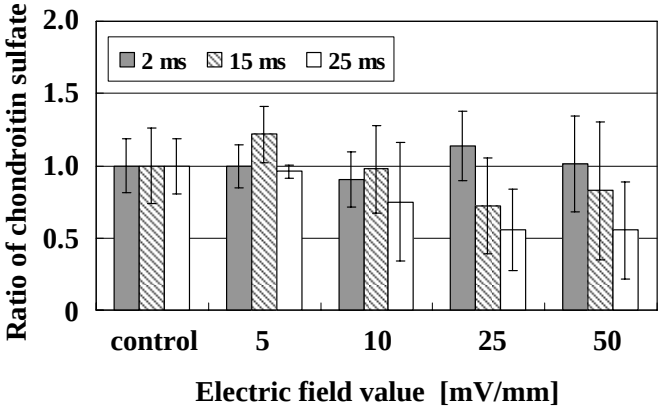


Figure 4 Ratio of chondroitin sulfate.

4. Discussion

Cell activity increased at 25 mV/mm electric field when pulse width was 2ms and cell activity increased at 5 mV/mm electric field when pulse width was 15ms, whereas cell activity decreased when pulse width was 25 ms. In the present study, a pulse wave was used for stimulation. A pulse wave has a period during which the voltage is at a constant value. The duration over which a constant voltage was applied, extended with longer pulse width. In a previous study, it was reported that stimulation by exposure to a direct current for 3 days caused cell death and decrease in the cell activity of chondrocytes[4]. Therefore it is possible that cell activity decreases with increasing pulse width. Furthermore the degree of decrease in cell activity increased with higher electric field value. These results show that short pulse width stimulates chondrocytes, and causes less damage than long pulse width. It is expected that cell activity can be stimulated by using a pulse width lower than 15 ms.

In a previous study, chondrocytes were stimulated by using an alternating current at frequency over 100 Hz [5]. In the present study, the frequency used was only 1 Hz, due to the similarity to a natural walking speed. A possibility of further study exists in using higher frequency in order to achieve higher level of stimulation.

5. Conclusions

- The costom-made chamber used in this study could stimulate chondrocytes with electric pulth field which was uniform and perpendicular to elctrode.
- Cell activity at 5 mV/mm elecric field increased when pulse width was 25ms, and cell activity at 25 mV/mm elecric field increased when pulse width was 2ms.
- Decrease in cell activity was caused with higher electric field values and longer pulse width.

6. References

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