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Single underwater spark discharge-induced shock wave used for physical gene transfer method

物理的遺伝子導入用単一水中火花放電誘起衝撃波

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1. Introduction

High pressure underwater shock waves which are generated by explosive process such as electrical discharge or microexplosive explosion have been successfully applied to a clinical use of extracorporeal shock wave lithotripsy[1-3]. Shock wave mediated gene transfection[4] has also attracted much attention as a physical method with high survival rate and high transfer efficiency.

In order to introduce the foreign substances into human primary cultured cells, we have used single shock wave generated by underwater spark discharge[5]. In this study, the acoustic characteristics of single underwater spark discharge induced shock wave were investigated using a polyvinylidene difluoride film-based hydrophone sensor which is known as a detector of underwater shock waves[6].

2. Experimental procedure

2-1. Shock wave generation

Single shock wave was generated by underwater spark discharge using an impulse voltage generation circuit. This circuit is composed of a charging circuit and a discharge circuit including a gap switch (GS). The basic operation of this circuit is as follows: The sinusoidal AC voltage (60 Hz) reduced by a variable autotransformer is boosted 150 times with a neon transformer. The boosted AC voltage is half-wave rectified by 40 series-connected-diodes. The boosted and rectified AC voltage is charged in five parallel-connected capacitors (each capacitance of 1700 pF) through a 50 kΩ resistor. The charging voltage is applied to the needle electrode in water through GS. In this experiment, the electrode distance fixed at 1 mm and 2 mm were used for the GS interval set to 2 mm and 3 mm, respectively.

The applied voltage, the discharge current, and the shock wave were measured by using a high voltage probe (Nisshin Pulse Electron, C0701) and a current transformer (PEARSON ELECTRONICS, 110), and a hydrophone sensor with rise time of 50 ns (Muller, Platte Needle Probe), respectively. These temporal waveforms were measured using a digital oscilloscope (Iwatsu, DS-5414).

2-2. Shock wave measurement

Figure 1 shows an illustration of the positional relationship between the needle electrode and the hydrophone sensor in the shock wave measurement. The tungsten needle electrodes (tip radius: 25 μm) were placed in a tank (61 × 61 × 85.5 mm) filled by pure water (conductivity 1 μS/cm). The needle electrodes were fixed at a height of 40.5 mm from the bottom of the tank. The hydrophone sensor was installed upward the needle electrodes and its position was adjusted precisely by using the three-axial ball slide stages (SIGMAKOKI) and a precision labjack (SIGMAKOKI). In this experiment, the distance between the electrode and the tip of hydrophone sensor was set to 6 to 12 mm.

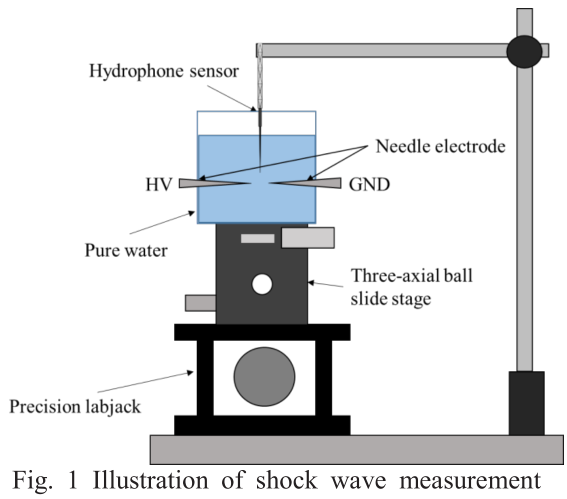

Fig. 1 Illustration of shock wave measurement apparatus.

3. Experimental results and discussions

3-1. Discharge and pressure waveforms

Figure 2 shows the discharge power and the pressure waveforms when the distance from the needle electrode to the hydrophone sensor is set to 10 mm. The results in the distance between needle tips of 2 mm and 1 mm are shown in Fig. 2(a) and 2(b), respectively. In these figures, the time of the peak power was set to 0 s. The discharge energy calculated from the power waveform of Fig. 2(a) and 2(b) were 0.429 J and 0.135 J, respectively. The each observed pressure waveform was composed

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mainly of positive pressure components and shows steep rise in pressure. The maximum values of the pressure shown in Fig. 2(a) and 2(b) were 11.9 MPa and 7.52 MPa, respectively. As can be seen from these figures, the each peak pressure was changed by changing the discharge energy, whereas, its waveform shape was unchanged. The full width at half maximum of pressure shape was 0.27 μs.

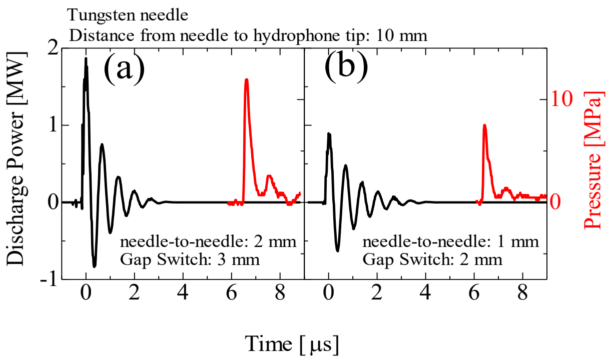

Fig. 2 Typical results of discharge power and the pressure waveforms in the distance between needle tips of (a) 2 mm and (b) 1 mm. The distance from the needle electrode to the hydrophone sensor was fixed at 10 mm.

3-2. Pressure gradient and impulse

The relationship between the waveform shape and gene introduction has been already investigated by several researchers. Mulholland et al. reported the effect of high stress gradient of the laser-induced stress wave (LISW) on the permeabilzation of the plasma membrane[7]. Kodama et al. showed that the impulse of shock wave was dominant factor for uptaking the molecules into the cytoplasm of human cells[8]. In this study, therefore, the pressure gradient and pressure impulse of underwater spark discharge-induced shock wave were calculated from the pressure waveforms.

Figure 3 shows the peak pressure dependence of the pressure gradient (dividing the peak pressure by the rise time) and the pressure impulse (integrating the pressure over time). The distance between the needle tips and the GS interval were 2 mm and 3 mm, respectively. The pressure gradient and pressure impulse values estimated from the LISWs reported in a literature[9] were also plotted in this figure. The calculated pressure gradient and pressure impulse values of underwater spark discharge-induced shock wave tended to be large in comparison with these results of LISWs. From these results, the single shock wave generated by underwater spark discharge even under 0.5 J in pulse energy has a superior ability in increasing with the permeabilzation of the plasma membrane required for gene transfer.

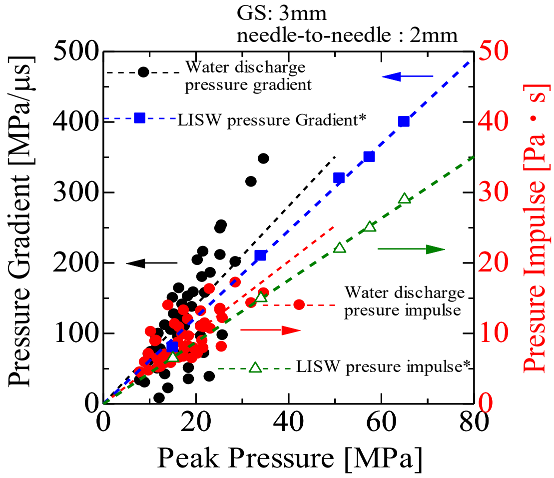

Fig. 3 Peak pressure dependence of the pressure gradient (closed black circles) and pressure impulse (closed red circles). The closed blues squares and open green triangles indicate the pressure gradient and pressure impulse estimated from the results of LISWs, respectively.

4. Conclusion

The acoustic characteristics of single shock wave generated by underwater spark discharge were investigated in detail. As a result, the generated shock wave consists mainly of the positive pressure component and has an ability used for gene transfer, even when the discharge energy is less than 0.5 J.

Acknowledgments

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