Acceleration of cell growth rate by plane shock wave using shock tube

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Summary. This paper describes effects of shock waves on cells to certificate the angiogenesis by shock wave (pressure wave) in the clinical application such as ESW (Extracorporeal Shock Wave). Especially, to investigate the effects of shock waves on the endothelial cells in vitro, the cells worked by plane shock waves using shock tube apparatus are observed by microscope. The peak pressure working on the endothelial cells at the test case is 0.4 MPa. After working shock waves on suspended cells, the disintegration, shape and growth rate (area per one cell and population of cells) are measured by image processing. It is found that the younger generation cells have small differences of shape index, and the growth rate of the shock-worked cells from 0 to 4h are clearly high compared with control ones. It is concluded that once shock waves worked, some of them are disintegrated, but the other has capacity to increase growth rate of cell culture in vitro. This preliminary result will be applied to fundamental investigations about shock wave stimulus on several kinds of cells in future.

1 Introduction

Recently shock wave phenomena in living tissues are being widely applied in the fields of medical and chemical engineering, such as extracorporeal shock wave lithotripsy, drug delivery systems ([2]), bioprocess for environmental protection and tissue engineering. In the field of tissue engineering, the bone therapy to regenerate the bone by extracorporeal shock waves shows the possibility for new therapy. In our previous investigation ([3]), it was found that the all of cells are not disintegrated by high shock wave (0.5 MPa) that has small duration time (50ms). So it is expected that the soft cells such as endothelial cells, cardio cells have a potential to regenerate or growth fast by using high pressure and short duration time from 1 micro s to 100 ms. But lately, Shimokawa et al. reported that extracorporeal focused shock wave (focused shock wave) accelerated cardiogenesys using low level ESW in the clinical application ([5]). They showed the typical photograph of angiograph, which indicates angiogenesis (or cardiogenesys) by extracorporeal focused shock wave. From this excellent study, it is found that the shock wave has ability to regenerate tissue cells. Concerning about the effects of focussed shock waves on living tissue cells and tissues, generally speaking, the focused pressure field in the ESW device is too complicated to estimate worked pressure level, and there should be cavitation bubble near the focused region. Then it is necessary to investigate these fundamental phenomena for effects of shock waves on cell disintegration, growth rate, regeneration.

In this paper, to investigate the effects of shock waves on the endothelial cells in vitro, the cells by plane shock waves in the shock tube are observed by microscope and the growth rate and others are measured by image processing.

2 Experimental apparatus and methods

Endothelial cells were taken from the lumen of pig's aorta by abrading with knife. These cells were cultured on the dish filled with DMEM (GIBCO) including 10 % FBS and 1 % PSN antibiotic mixture in CO_2 incubator under the proper condition (Temperature 310 K, humidity 100%, 5% CO2). Subculture is proceeded by checking 70

As mentioned in the introduction, the key point of this investigation is to use shock waves by simplifying the pressure fields working on the cells. Shock waves to apply endothelial cells are generated by so-called shock tube (Fig.1) . This shock tube has a novel technique that has no diaphragm to generate plane shock waves easily. The feature of the shock tube apparatus is that (1) the shape of the wave is plane and (2) the duration time is long (several 10 ms) compared with laser shock wave and focusing shock wave. As for the apparatus, after several valve operations, the plane shock wave is propagating into the test case located at the wall end in Fig.1. In this test case, the cells were suspended in the cultured liquid and the top surface is wrapped with thin film to prevent the dropping liquid. The important point to set up the test cells is not to include gas bubbles in the wrapped test case, otherwise the effects of bubbles on the cells and pressure fields should be complicated.

Generally, there are two boundary conditions for endothelial cells in the shock wave apparatus. One is the effects of shock waves on suspended cells, the other is fixed cells on the culture dishes. In this paper, only the suspended cells are discussed because setting up the cells is simple operation. . The experimental conditions for shock waves are pressure ratio in the apparatus is 4, Mach number 1.4 in air, shot number is from 1 to 5. Here interval time for each shot of shock wave is 10 - 30 seconds. Figure 2 shows pressure history at the test case. From this result, the peak pressure in the pressure history in air

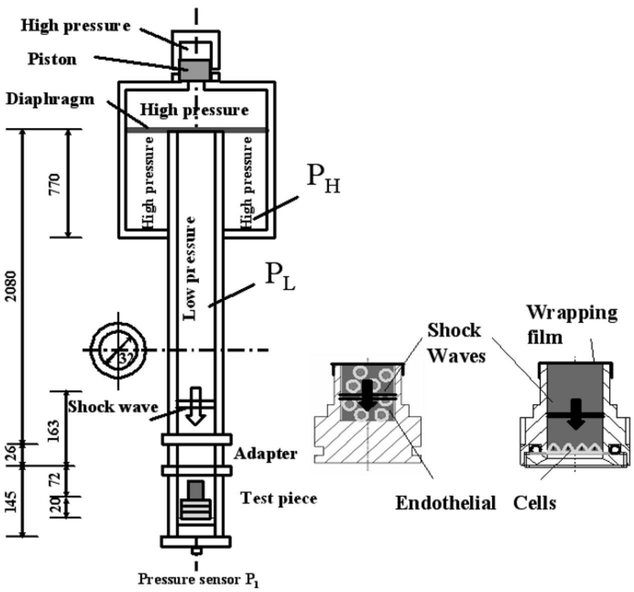

Fig. 1. The schematic of diaphragmless shock tube and test piece with film for shock

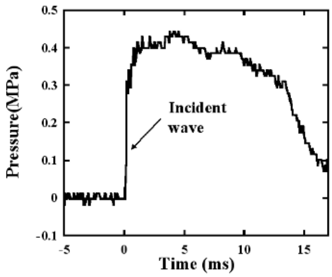

Fig. 2. Pressure history at tube end

at the test case is 0.4 MPa. The pressure sensor is located at the bottom of the shock tube (tube end), so the pressure of incident shock wave in air is estimated to be 0.2 MPa by considering the wave reflection. Then the pressure of incident shock wave in water is estimated to be about 0.4 MPa. The thickness of the shock wave in water is not clear, but usually considered to be several μm. After working shock waves on suspended cells, the disintegration, shape and growth (population of cells and area of cells) are investigated. Every conditions, there are at least one control and one worked cells.

3 Experimental results

3.1 Population of endothelial cells after shock waves

Before observing the deformation and growth of cells, the disintegration rate by shock wave should be checked because the survival number of cells is very important factor for estimating growth rate. The results has already shown that the maximum disintegration of endothelial cells are above 20% at shot number 5.

Concerning about the cell population in the image taken by phase contrast microscopy, it should be taken account of disintegration rate of cells after shock wave with shot number as follows; .

$$N_n = \frac{NS_n}{NS_0} \times (1 - \alpha) \tag{1}$$

Here N_n, N_0 means population of cells at n hours, and population of cells at 0 hour respectively. And N_n is normalized cell population. Figure 3 shows dimensionless population of cells history with shot number. From Fig.3, it is found that according to shot number the population of cells is increasing compared with control cells. In this figure, the population of cells can be counted in the image data, so the error bar and probability is not shown here. It is found that the growth rate of the shock-worked cells from 0 to 4h are clearly high compared with control ones. Especially in case of passage number 3, the population is rapidly increasing by 5 shots shock waves. Concerning about effects of the passage number on the population, the population of several hours of the passage number 3 is about twice as that of passage number 6. This fact also shows that the younger cells have the ability to increase themselves well as mentioned above.

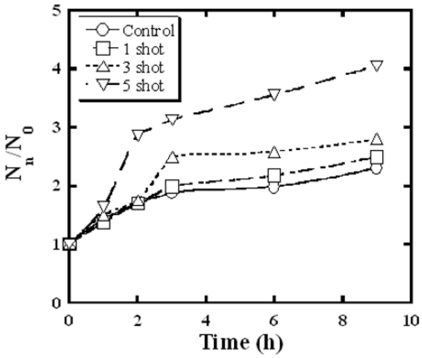

Fig. 3. Population of endothelial cells on the culture dish with shot number

3.2 Gene expression after shock waves

To confirm the capability of acceraltion of cell growth by shock wave, the gene expression is being tested test for these cells by PCR (Polymerase Chain Reaction) method. Quantity of gene expression is estimated as cycline D2

3.3 Discussion

From the above results, it should be discussed about the mechanical stimulus on the cells. Generally the thickness of shock wave is about several μ, this scale is almost the same scale as the cell itself. This scale is very importtant becuase the profile of mechanical stimulus is changed rapidly in the cell compared with other static stimulus.

Using this considerations, then following assumption can be considered. Once the shock wave applied the cell membrane, the cell membrane or the protein on the membrane senses the pressure gradient (normal stress and shear stress) more sensitively than that in case of quasi-static pressure, then the signal from the membrane protein is transmitted inside the cell.

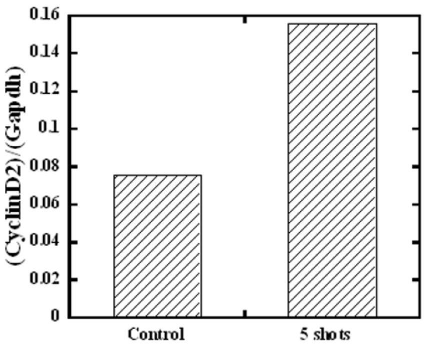

Fig. 4. Effect of shock waves on gene expression of endothelial cells

4 Analysis of propagating shock wave to cell model

4.1 Modeling of propagation shock wave to cell

The computational model is composed of following two stages; (1) Underwater shock wave propagates in the water region (fluid dynamic stage), (2) Propagating shock wave works to the hemisphere cell on the wall (solid dynamic stage). To connect these two computational stages, one dimensional visco-elastic wall model for the fluid-solid interface. The input pressure condition to work this model is important, then the rise time of the pressure wave (shock wave) is defined to be from 0.2 to 12 *ns*. In this computation, the step pressure with maximum pressure 0.2MPa is worked.

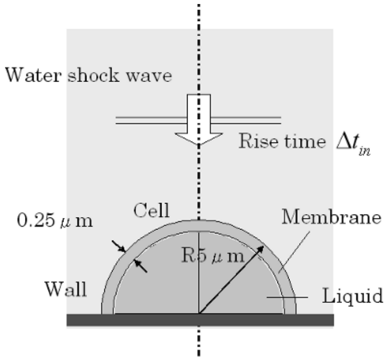

Fig. 5. Model of shock wave propagation to adhesive cell

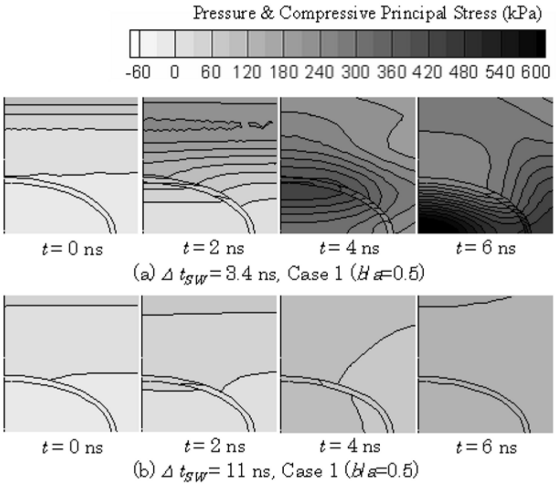

Fig. 6. Pressure and stress wave propagation between fluid and cell

4.2 Computational results and discussion

Figure 6 shows pressure contour after working shock wave to the cell model. Figure 6(a) shows the contour in the case of rise time 3.4 ns, Fig.6(b) shows that in the case of 11 ns. From this, it is cleary found that pressure gradient nrat the cell membrane for the short rise time is larger than that for the long rise time. Then it is considered that the shorter the rise time is, the larger shear stress gradient becomes in the range of 0.2-12 ns. The short rise time has possibility to give large stimulus to the cell membrane or cell.

5 Conclusion

To investigate the effects of shock waves on the endothelial cells in vitro, the cells by plane shock waves were observed. It is concluded that once shock waves worked, some of them are disintegrated, but the other has large ability to increase growth rate of cell culture in vitro. The growth of cells by plane shock waves is also confirmed by the fact that activated gene expression is enhanced. In addition to this, the shorter rise time is, the stimulus to the cell by pressure wave (shock wave) becomes.

In future works, effects of shock waves on the fixed cultured cells in the test case should be done to compare this work, because the normal endothelial cells are fixed on the tissues. And effects of pressure wave forms on the growth rate also should be done because it is important to distinguish the effects of pressure fields.

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