SINGLE CELL SURGERY WITH MONODISPERSED MICROBUBBLES GENERATED BY A PULSED DISCHARGE OF MICROELECTRIC KNIFE

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ABSTRACT

We have successfully operated enucleation of oocyte by using microelectric knife without any thermal collateral damage. Minimally-invasive cellular-scale ablation was achieved by the mono-dispersed microbubbles which were generated by a pulse discharge of microelectrode. The discharged output power and conductive area of microelectrode were controlled by glass shell insulation around the copper microwire. A small space which is called “bubble reservoir” between the wire and glass tip contributes to stabilize the electric discharge and directional bubble generation.

KEYWORDS

Micro-nano bubble, electrically-induced, enucleation

INTRODUCTION

Electric knife is one of indispensable surgical devices and it was used in wide surgical operation. This technique, unlike laser technology, has not been remarkably improved since it has invented several decades ago, while manipulation and processing technology of microorganisms or cells become important subjects with the development of the MEMS, gene technology and neurology [1]. Therefore, low in cost, high in resolution and non-intrusive ablation under medium is highly required. Recent development of the microelectric knife succeeded in its miniaturization [2] however unavoidable thermal collateral damage at the fracture cross-section damages the protein binding of cell. For the present study, we utilized a novel phenomenon of mono-dispersed electrically induced bubbles for cell ablation. Figure 1 classified the conventional cell ablation techniques compared to the proposed system. Compare to the other techniques, proposed microelectric bubble knife has advantages in terms of cost and limited collateral damage.

EXPERIMENT

Figure 2 shows the concept of the microelectric bubble knife. Mono-dispersed directional microbubbles generated by the oscillation of air-liquid interface at the edge of the probe can ablate a surface of cell using microjet phenomenon produced by the microcavitation. The electrical circuit of conventional electric knife was modified with non-inductive resistance, and which enabled the cellular-scale ablation as shown in Figure 3. The applied output power was in the range of 0.1-1.0 W at frequency of 450 kHz. Figure 4 shows the process flow to fabricate the microelectric bubble knife using the glass puller with a copper microwire. After several preliminary experiments, it was confirmed that the coating is not a sufficient method to insulate the electrode perfectly. It is necessary to have a glass-insulated shell protecting the electrode by preventing unnecessary water invasions. To produce such an electrode, the insulated layer and a wire were fabricated simultaneously to produce a knife for perfect insulation. To produce such an insulated microelectrode, the glass capillary tube (50 μL) and a copper wire with a diameter of 30 μm and a silver paste were used.

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<th>Resolution</th>
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<th>Conventional Glass Capillary</th>
<th>Current Electric Bubble Knife</th>
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Figure 1. Classification of technologies for cell surgery

Figure 2. Concept of cell ablation by micro/nano bubbles generated by electric knife (a)concept view of microelectric bubble knife and bubble reservoir (b)Mechanism of ablation (c) Electrical circuit of enucleation of cell

Figure 3. Electrical circuit of microelectric knife
Then the glass puller (P-1000IVF) was used to pull the glass capillary. The copper wire was passed through the glass tube and was set to the glass puller. After applying the programmed thermal input, the tube and the wire can be disconnected. Then the silver paste was stuffed to the end of the tube to connect to the power supply of the electric knife. Due to the viscoelasticity of glass insulation, glass can be extended further than copper and which produced a space called “bubble reservoir” which make stabilize the electric discharge.

Figure 5 shows high-speed camera photos of the phenomenon of a line of mono-dispersed microbubbles generation. This peculiar phenomenon of directional bubbles which can ablate the cell surface contributes to the positioning accuracy of the ablation.

Next, the ablation test of actual cell (bovine oocyte) was carried out. The confocal microscope image of the fluorescent ooplasm and zona pellucida dyed by rhodamine B confirmed that the microelectric bubble knife can process the cell membrane and control the depth of ablation with limited damage successfully. Figure 6(d) shows the result of the evaluation of the width of the ablation area as a function of the distance between the cell and electrode. It was confirmed that the larger distance provide the smaller width of the ablation. The diameter of the exit part of the microelectrode is approximately 10 μm and the width of the cone-shape bubble boundary reduced below 10 μm after the distance D becomes 10 μm.

RESULTS AND DISCUSSIONS

Finally, this technique was applied to the enucleation of oocyte which is one of the most complicated processes in the cloning technique. Figure 7(b) shows the conventional manual operation of enucleation by a glass capillary and which is difficult task requiring 3D dissection by skilful operators. Figure 7(a) shows the first trial of the enucleation of oocyte by the microelectric bubble knife. The target of the nucleus was stained by Hoechst 33342.
The experiment was carried out in the half dark field with fluorescent light. It was confirmed that there is no thermal collateral damage using microelectric bubble knife. The width of the ablation region was successfully reduced to more than a half. A nucleus dyed by Hoechst was removed successfully with limited damage region (≈ 5 μm) which is difficult task for manual operation with glass capillary. Figure 7 (c) shows the comparison of the ablation region after the enucleation and it was confirmed that the ablation area by microelectric bubble knife is about a half of that by manual operation with glass capillary.

After the first trial of the enucleation test, we aim to the enucleation process without using toxic fluorescent dye to increase the production rate and to obtain the minimally invasive operation. We use polar body as a target of the enucleation without using any fluorescent dye. Generally the polar body exists next to the nucleus of the oocyte, when the oocyte is in the matured stage II. Therefore removal of the polar body and ambient area complete the enucleation sufficiently. To confirm the successful of enucleation, we simply separate the ablation part to the other dishes where the cell was stained by Hoechst for confirmation of the enucleation. Also, it was found that the membrane of the ooplasm of oocyte should not be damaged during the operation, and more importantly the membrane should be regenerated to keep the spherical shape even after the enucleation process. To obtain such a procedure, it is highly required to evaluate the direction of shooting of electric-bubble knife. Figure 8 shows the proposed tangential direction of enucleation by microelectric knife. To obtain the minimally invasive operation, the enucleation was carried out along the tangential line around the polar body. Figure 9 shows the new method of enucleation in the bright field. To obtain minimally-invasive operation and to keep the membrane of the ooplasm in good condition, the ooplasm was simply thrust out by pushing the oocyte by electric bubble knife operated by the manipulator after piercing 3D hole in the oocyte by microelectric bubble knife. It was successfully operated the removal of polar body of oocyte in the bright field. It was confirmed that the ooplasm after the operation keep the spherical shape which indicate the ooplasm is in good condition. On-going research is carried out to increase the production rate after the operation.

CONCLUSIONS

For the present study, we proposed microelectric bubble knife by using a phenomenon of a directional line of mono-dispersed micro-nano bubbles between the electrodes. It was confirmed that the cell was fabricated with a few μm-order resolution. Enucleation of oocyte was also successfully operated. The first trial enucleation test was carried out in the half dark-field with fluorescent light, it was confirmed that the ablation area by microelectric bubble knife is about a half of that by manual operation with glass capillaries. In the case of the minimally invasive enucleation in the bright field without using fluorescent dye, we have successfully removed the polar body with ambient part by tangential direction of shooting of electric-bubble knife.

On-going research is carried out to increase the production rate of bovine oocyte enucleated by the microelectric bubble knife. This microelectric bubble knife is minimally-invasive and simple structure. It is easy to assemble to general medical instrument such as endoscope and expected to be used in-vivo environment for more practical use.

This low cost microelectric bubble knife has possibilities to extend to fabricate any objective material under various environments and contribute to a new top-down fabrication method in the micro-nano bioengineering field.

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REFERENCES


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