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Electrochemical treatment (ECT) of cancer is a promising new method which delivers direct current (dc) into tumor tissue by inserting electrodes (anodes and/or cathodes) to induce tumor regression. The purpose of this study is to evaluate the effectiveness of ECT and tissue responses to anode and cathode. **Methods:** (1) Tumor tissue responses to ECT: 1×10^6 rat breast cancer cells (MTF-7) were injected subcutaneously into the right fat pad of the mammary gland of a Fisher 344 female rat. While the tumors were grown to 2 × 2 × 2 cm, 60 rats were divided into 6 groups and underwent ECT with doses of 0, 5, 10, 20, 30, and 40 coulombs (C), respectively (9 in each group except 12 in 5 and 10 C groups). Two platinum electrodes were inserted at a 1-cm spacing into the tumor. Three tumor samples in each group were removed at 0, 24, and 48 hrs post-treatment for light microscopy and at 0 hour for electron microscopy. (2) Normal muscle tissue response to ECT: 9 Fisher 344 female rats were divided into 3 groups: anode, cathode, and both anode and cathode insertion in right thigh muscle (3 in each group) with 10 C applied. **Results:** There are distinct changes to the cellular structure of the treated tumors. The changes ranged from blurred to complete loss of cellular outlines. An abrupt transition from treated tumor to viable tumor indicated that there is a definite effective treatment area, based upon the dose applied. The area adjacent to the cathode exhibited minor swelling, while dehydration was observed around the anode. Also, We observed disruption in the treated muscle tissue, either with cathode, anode, or both. **Conclusions:** (1) ECT is effective for cellular disruption of the affected tissue; (2) Both anode and cathode can damage and destroy cell structures; (3) The effects of ECT are non-specific.

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CONTENTS

INTRODUCTION

Electrochemical treatment (ECT) of cancer is a promising new method which delivers direct current (dc) into tumor tissue by inserting electrodes (anodes and/or cathodes) to induce tumor regression. Dr. Björn Nordenström of Sweden was the first in recent years to utilize dc for treating human lung tumors (1,2). This method was later used to treat breast cancer (3). The most impressive data, consisting of 4081 cases from 818 hospitals in China, were reported by Dr. Xin Yu-ling. ECT has become an alternative method for cancer treatment in China and is being used in about 1000 hospitals (4.5). Even though ECT has been applied in clinical studies of different kinds of tumors and has shown positive response, there has been almost no systemic research in this field. Little has been done on the basic research, such as effectiveness evaluation, dose-response relationship, and cytotoxicity. The biological mechanism of ECT is also poorly understood. The treatment methods are arbitrarily. For instance, in some European studies, the anodes were placed in tumor tissue and the cathodes in normal tissue (1), while in some Japanese and Chinese studies, both anodes and cathodes were placed in tumor tissue (6-8). The purpose of this study is to evaluate the effectiveness of ECT and tissue response to anode and cathode by investigating the ECT induced pathological changes of rat beast cancer tissue and normal muscle tissue.

MATERIALS AND METHODS

ECT Instrument:.

A four-channel instrument (BK92A; Beijing University of Aeronautics and Astronautics, Beijing, China) is used for this study. This is a computer-based dc power supply where the maximum voltage (V), current (mA), electrical charge (C), and time (min) may be set. The treatment may be set to a constant-voltage or a constant-current mode. Since clinical studies indicate that treatment at high voltages can cause pain to the patients, the constant-voltage setting is used to prevent this complication. The instrument has the capability of slowly "ramping" up to the set voltage (or current, if the constant-current mode is used) to prevent shocking the patient. Open-circuit and short-circuit resistance warnings and the rate of voltage increase may be set individually.

Electrodes:

The 24 gauge (~0.53 mm diameter), pure platinum wire (Model 9924-F50) was purchased from Thomas Scientific, Swedesboro, New Jersey. The wire was cut into 6-cm fragments and the tips were sharpened to facilitate insertion. Catheters were used for initial tumor penetration since the platinum electrodes were too soft for rat skin. The electrodes were cleaned and sterilized in 70% EtOH prior to use.

Rat MTF-7 breast tumor cells:

Rat MTF-7 breast cancer cells were obtained from the M. D. Anderson Hospital and Tumor Institute, The University of Texas System Cancer Center, Department of Tumor Biology and tested for viruses (Microbiological Associates, Inc.). The cells were grown *in vitro* in MEM Eagle with Earle's Salts and L-glutamine (200 mM). The solution was supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin (10,000 units/ml penicillin G and 10,000 μg/ml streptomycin sulfate in normal saline). When the cells reached approximately 80% confluence, they were collected and suspended in PBS for injection. The final concentration for the injection was 10^6 cells/0.25 ml.

Tumor model creation:

Fisher 344 female rats, with a weight range of 145-156 grams, were used in this study (source: Charles River). 0.25 ml of the cell suspension (10^6 cells) was injected subcutaneously beneath a nipple in the right chest of the rat. When the tumor reached to approximately 20 mm in diameter, the animal was ready for ECT.

Animal groups:

A total of sixty-nine rats were used in this study. Sixty rats were used to determine the effects of different doses used on tumor tissue. The remaining nine rats were used to examine the effects of ECT on normal muscle tissue. For tumor tissue responses to ECT, 60 rats were divided into the following groups: control, 5 C, 10 C, 20 C, 30 C, and 40 C. The 5 C and 20 C groups had 12 rats in which 9 were for light microscope (LM) studies and 3 were for transmission electron microscope (TEM) studies, other groups had 9 rats for each and only underwent LM studies. The control groups were shared for both LM and TEM studies. For normal muscle tissue response to ECT, 9 rats were divided into 3 groups: anode insertion, cathode insertion, and both anode and cathode insertion. Three rats for each group.

ECT procedure:

Before ECT, rats were anesthetized intraperitoneally with a 3:1 Ketamine:Xylazine cocktail. Plastic plates with slots were used to lift the tumor away from the body. The eyes of the rats were protected from dehydration with an ophthalmic antibacterial ointment. Once the rats were anesthetized, two platinum electrodes were inserted vertically (perpendicular to the plane of the body) into the tumors at a 10-mm spacing and connected to the anode and cathode of the ECT instrument, respectively. Additional parameters were configured as follows: 8 V, a maximum of 80 mA, and a maximum treatment time of 120 min. A maximum of 4 rats can be treated at the same time (Figure 1). For normal muscle tissue response to ECT, anode, cathode, or both anode and cathode were inserted in right thigh muscle and 10 coulombs were applied. While anode or cathode was inserted in the muscle, the other electrode was inserted in the tumor. While both anode and cathode were inserted in the muscle, the space between two electrodes was 10 mm.

Sample collection:

Upon completion of the treatment, tumor samples were removed immediately, 24 hours, and 48 hours following treatment for LM studies. For TEM and muscle response studies, the samples were removed immediately following ECT. Three samples were collected at each time point from each group and each rat provided only one sample. Light microscopy samples were prepared with standard H&E staining procedure. Transmission electron microscopy samples were prepared following a 4% paraformaldehyde/ 1.7% glutaraldehyde procedure.

RESULTS

Naked eye observation:

During ECT, the color turns to black in the anode area and reddish in the cathode on skin of tumor site (Figure 2) while yellow in the anode and white in the cathode inside of tumor. Bubbles were observed around the cathode (Figure 2). The tumor tissue shrank and presented caseation in anode area and congested and swelled in cathode area. Twenty-four hours after ECT, the entire tumor shrank and a scar is formed on the tumor surface (Figure 3). Seventy-two hours later, the tumor fell off, leaving a open wound at the previous tumor site (Figure 4). The wound gradually recovered by the formation of skin over the treatment area in a few days after ECT.

Light microscope (LM):

Our observations indicate that there are distinct changes to the cellular structure of the treated tumors. Figure 5 shows control rat MTF-7 breast cancer tissue. In the anode area, the nuclei shrank and the cytoplasmic structure disappeared (Figure 6). In the cathode area, the cell swelled and the interface among cells blurred (Figure 7). The extent of cell structure disruption was related both to the condition of the tumor at the time of treatment and the dose applied during treatment. These changes ranged from blurred to complete loss of cellular outlines. An abrupt transition from treated tumor to viable tumor indicated that there is a definite effective treatment area, based upon the dose applied (Figure 8, 9). The area adjacent to the cathode exhibited minor swelling, while dehydration was observed around the anode. Also, we observed disruption in the treated muscle tissue, either with cathode, anode, or both (Figure 10-12).

Transmission electron microscope (TEM):

Figure 13 shows control rat MTF-7 breast cancer cells. After 5 C ECT, cells shrank, mitochondria swelled mildly and the crest disappeared, polysome disaggregated, lysosome distended, and nuclear chromatin aggregated focally in anode area (Figure 14) while cells swelled and nuclei vacuolized in cathode area (Figure 15). At a higher level 20 C, plasma membrane burst and the distended organelles escaped in both anode and cathode areas (Figure 16, 17). Microscopic studies reveal morphological changes corresponding to inhibition of cell proliferation at ECT.

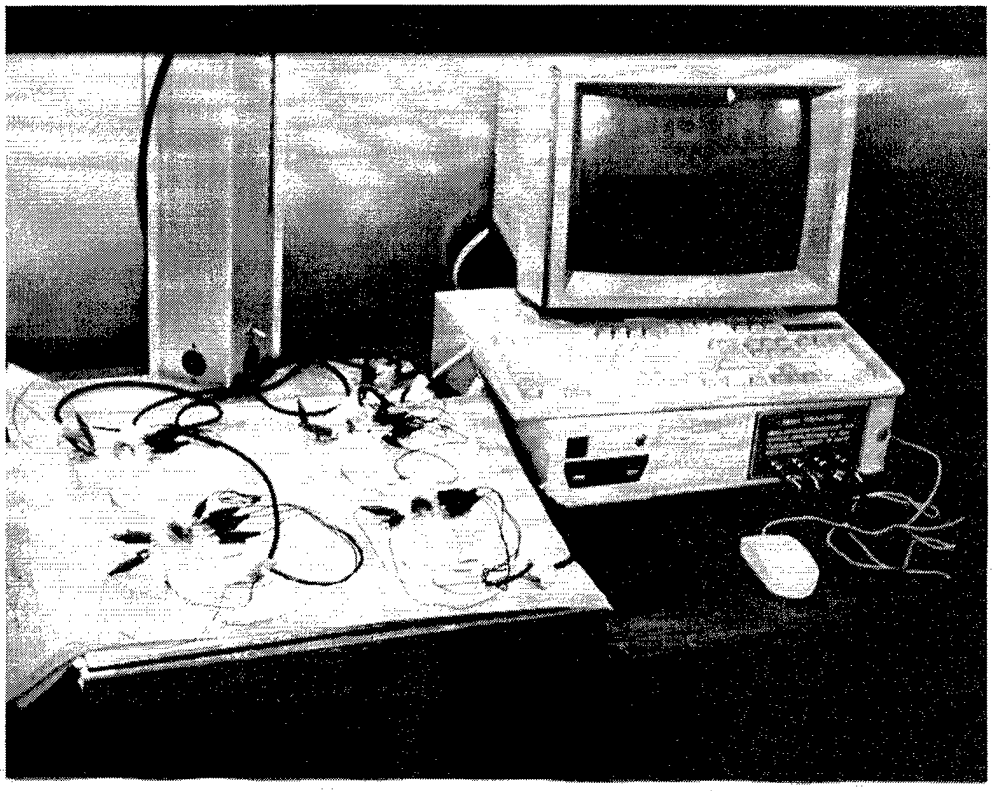

Figure 1. The four-channel ECT system.

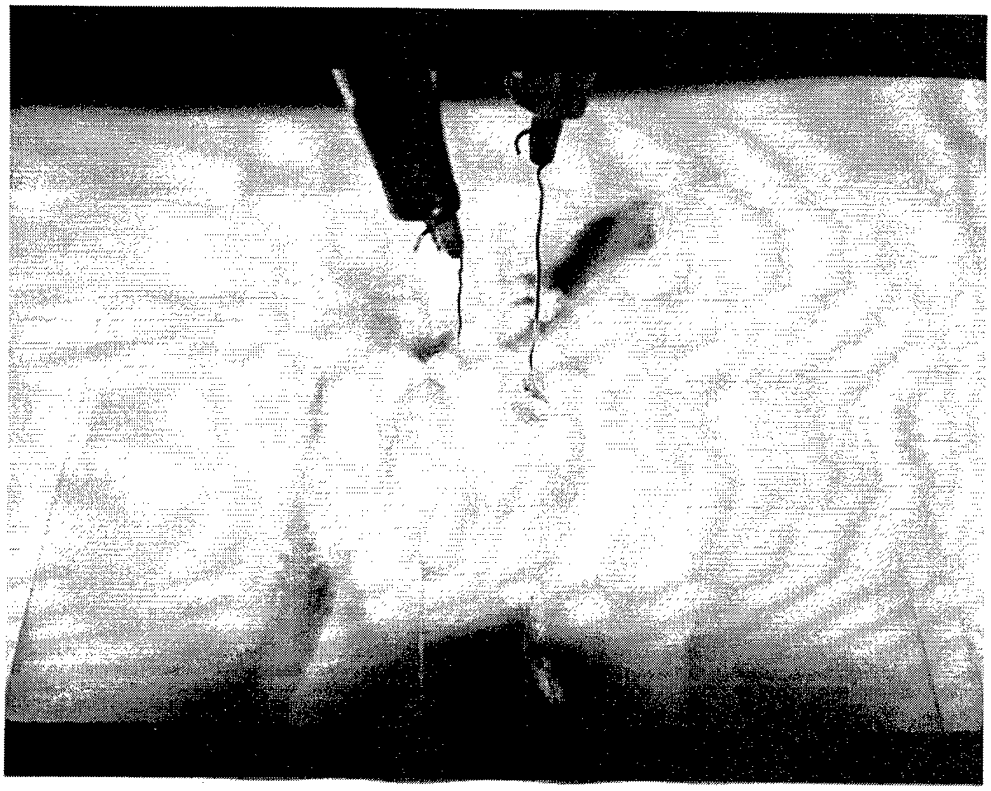

Figure 2. During ECT, the red clip was connected to the anode of the ECT system while the black one was connected to the cathode.

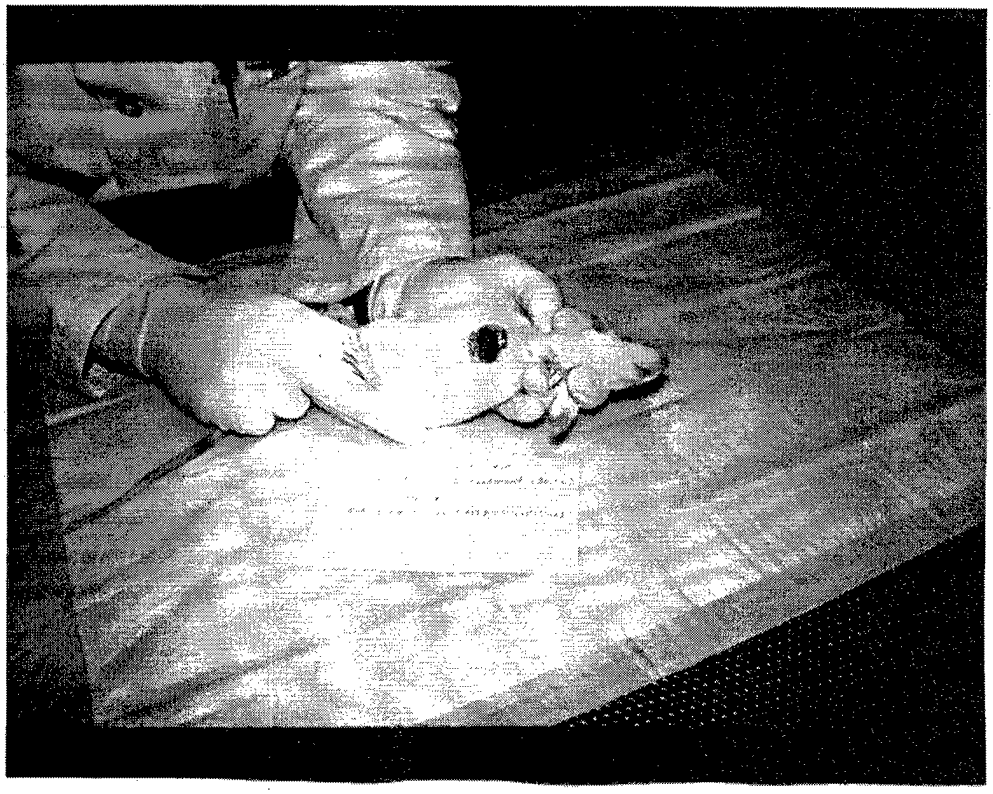

Figure 3. Twenty-four hours after ECT.

Figure 4. Seventy-two hours after ECT, the tumor fell off.

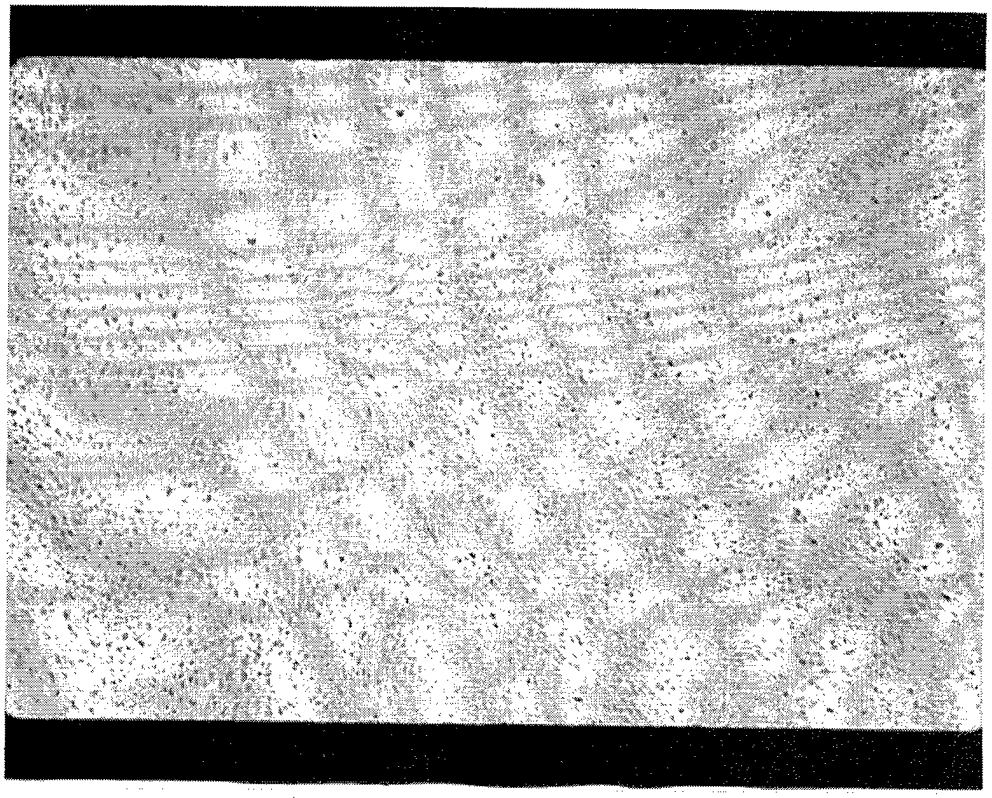

Figure 5. LM: The MTF-7 rat breast tumor tissue before ECT (Control). H&E staining.

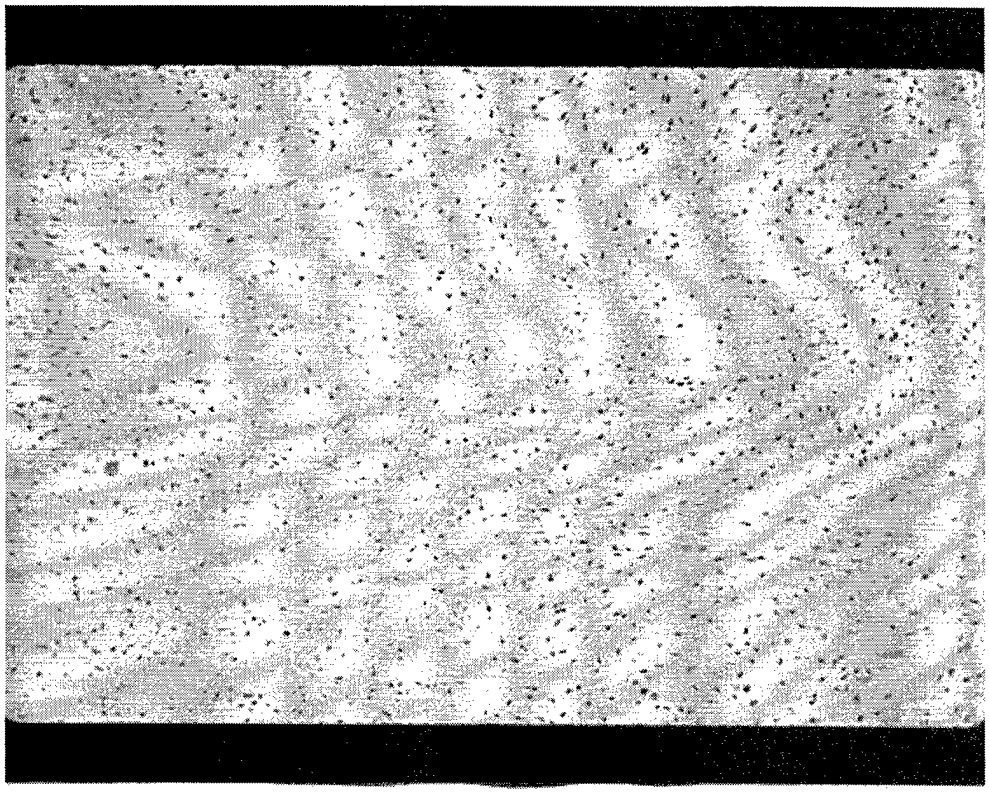

Figure 6. LM: The tumor tissue in anode area immediately after ECT. H&E staining.

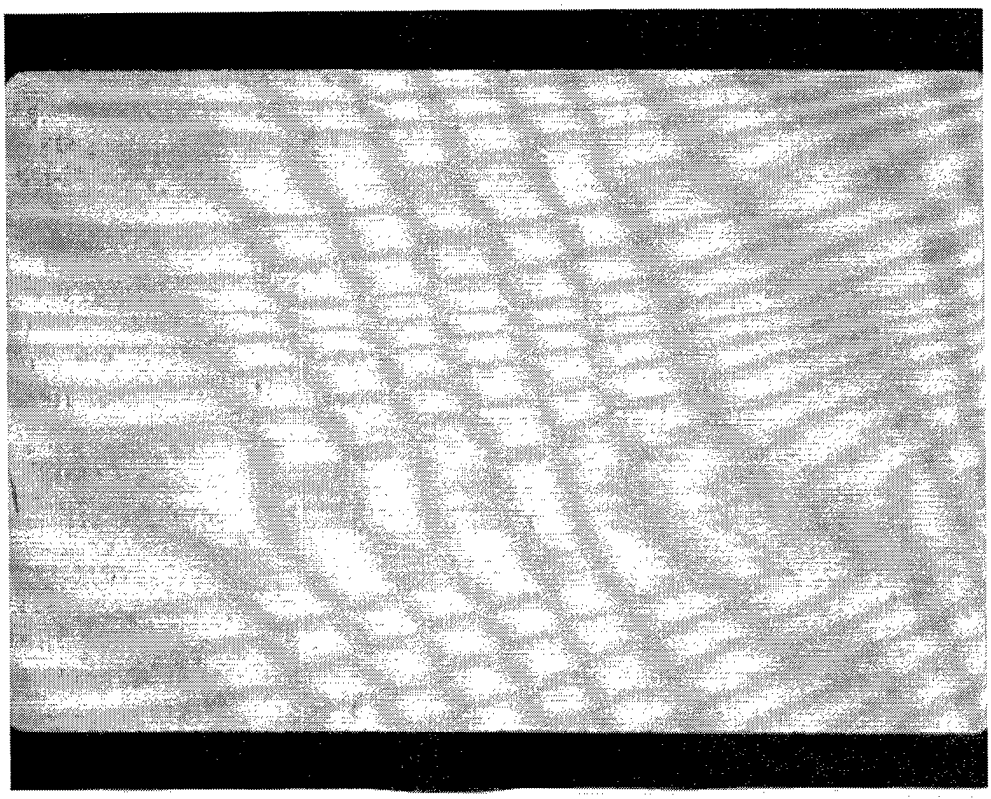

Figure 7. LM: The tumor tissue in cathode area immediately after ECT. H&E staining.

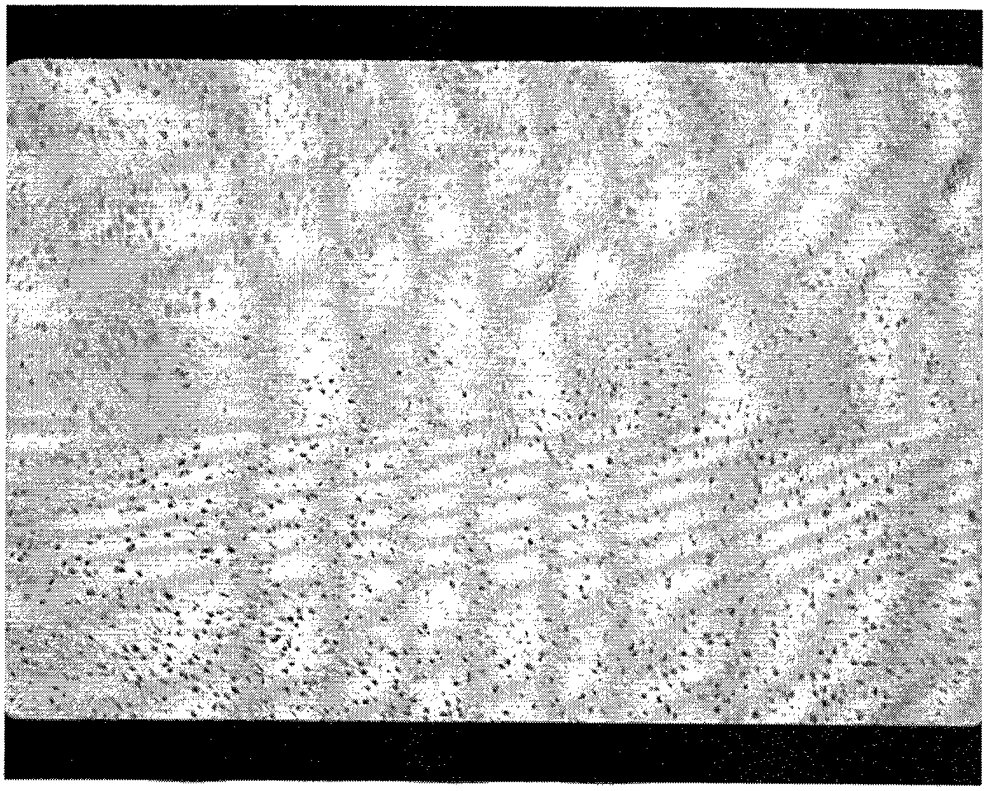

Figure 8. LM: The borderline between anode area and normal tumor tissue. H&E staining.

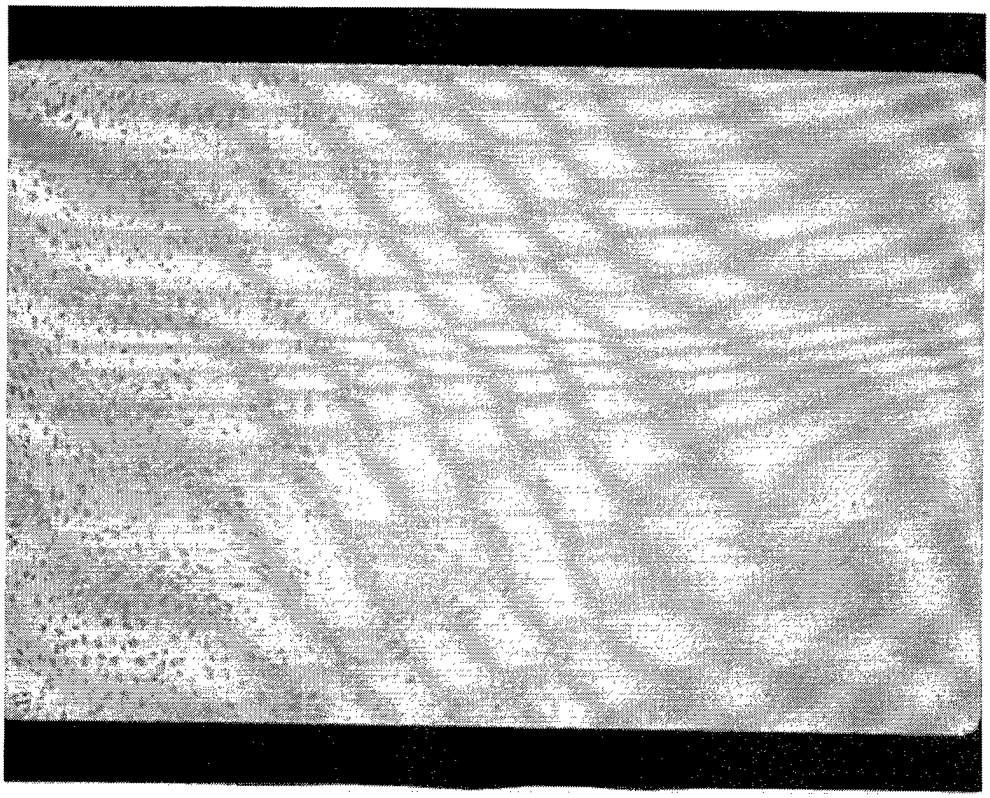

Figure 9. LM: The borderline between cathode area and normal tumor tissue. H&E staining.

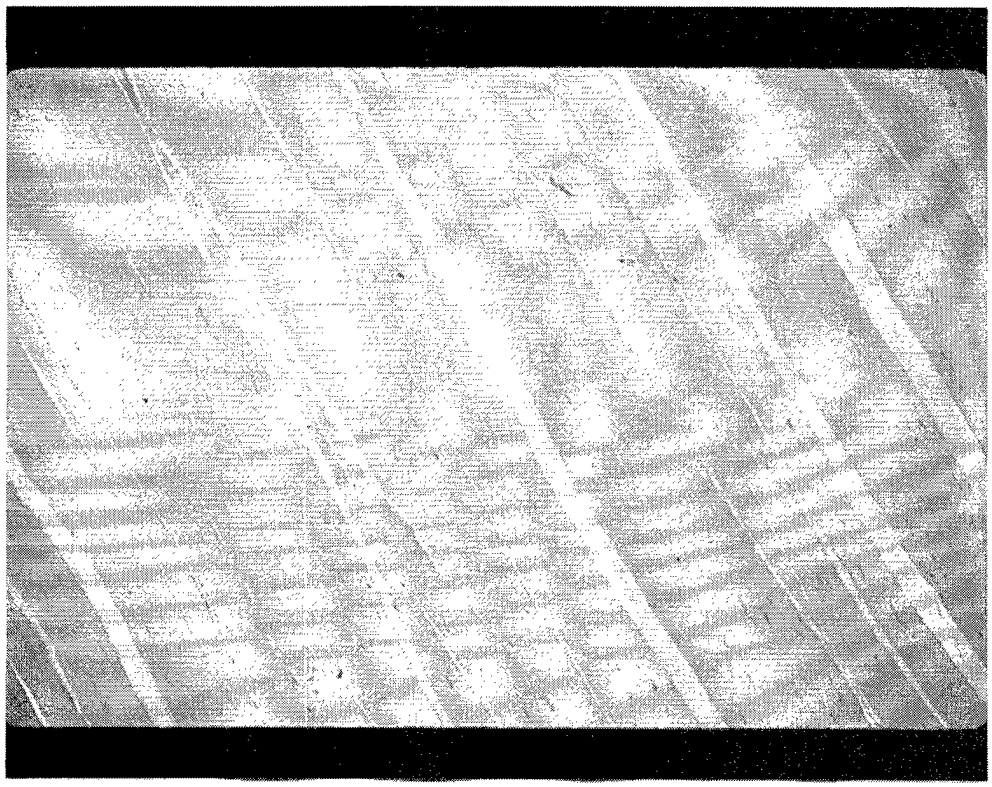

Figure 10. LM: Normal striated muscle.

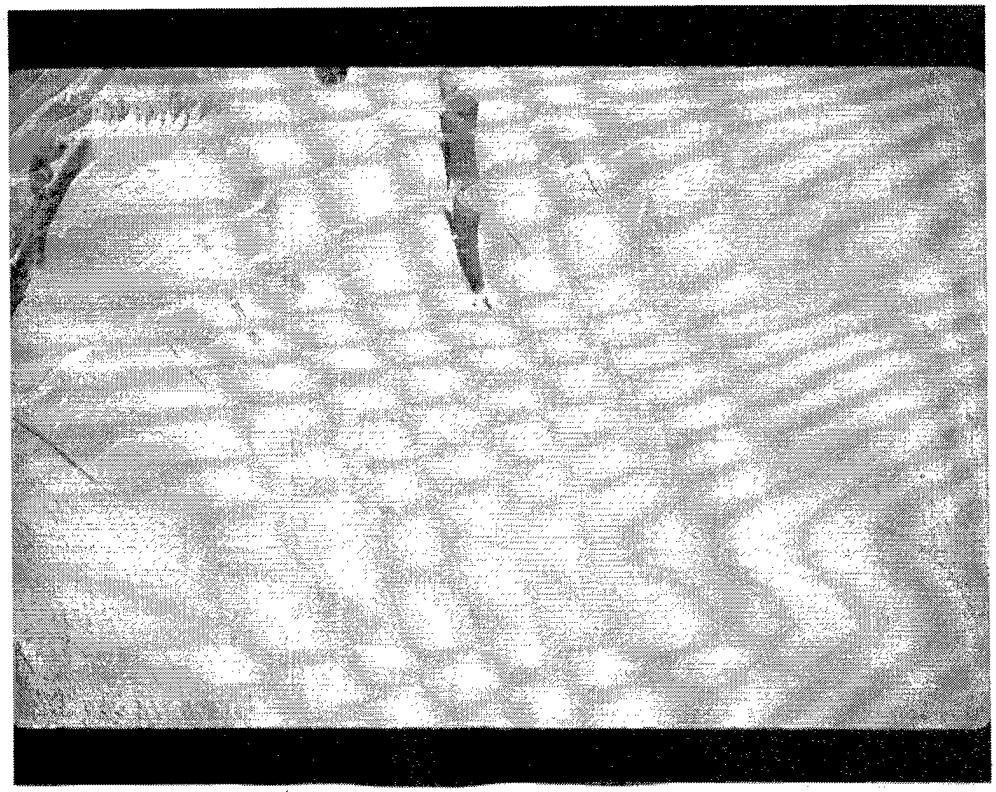

Figure 11. LM: The striated muscle in anode area immediately after ECT.

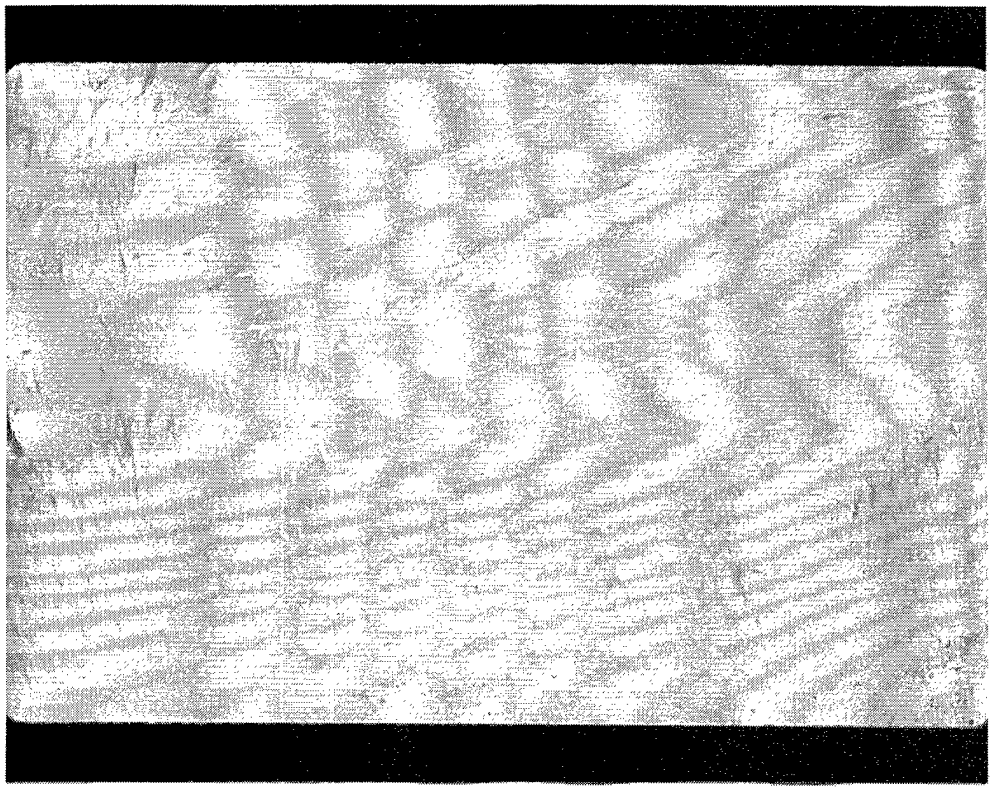

Figure 12. LM: The striated muscle in cathode area immediately after ECT.

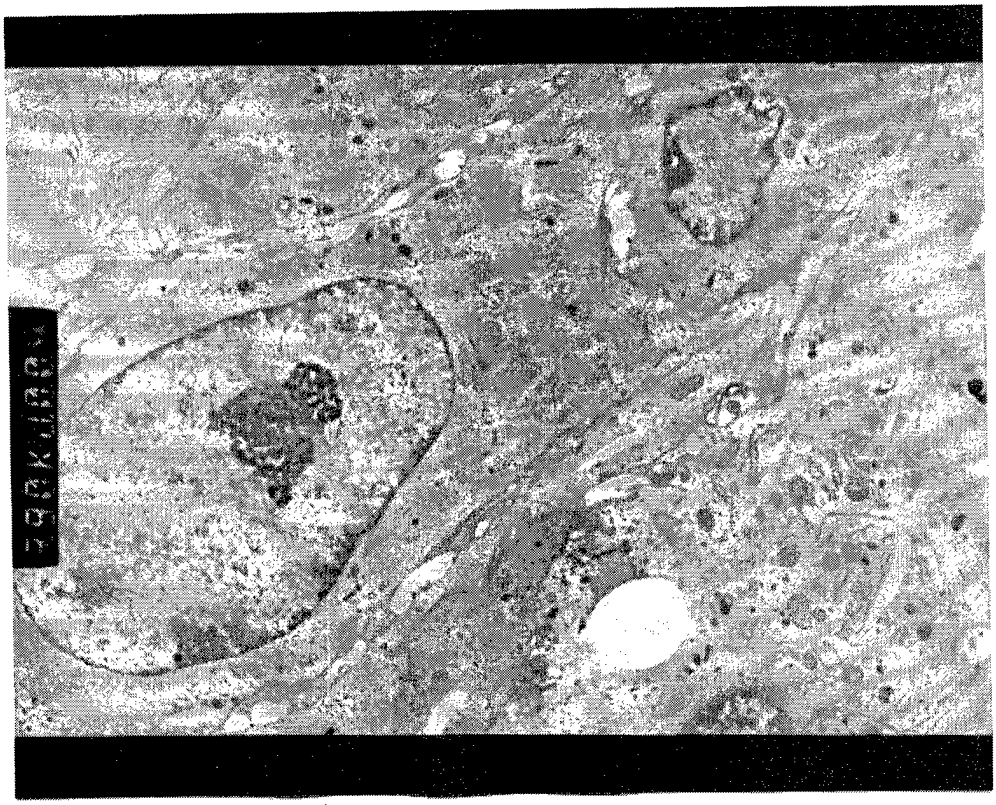

Figure 13. TEM: The MTF-7 rat breast tumor cells before ECT (Control).

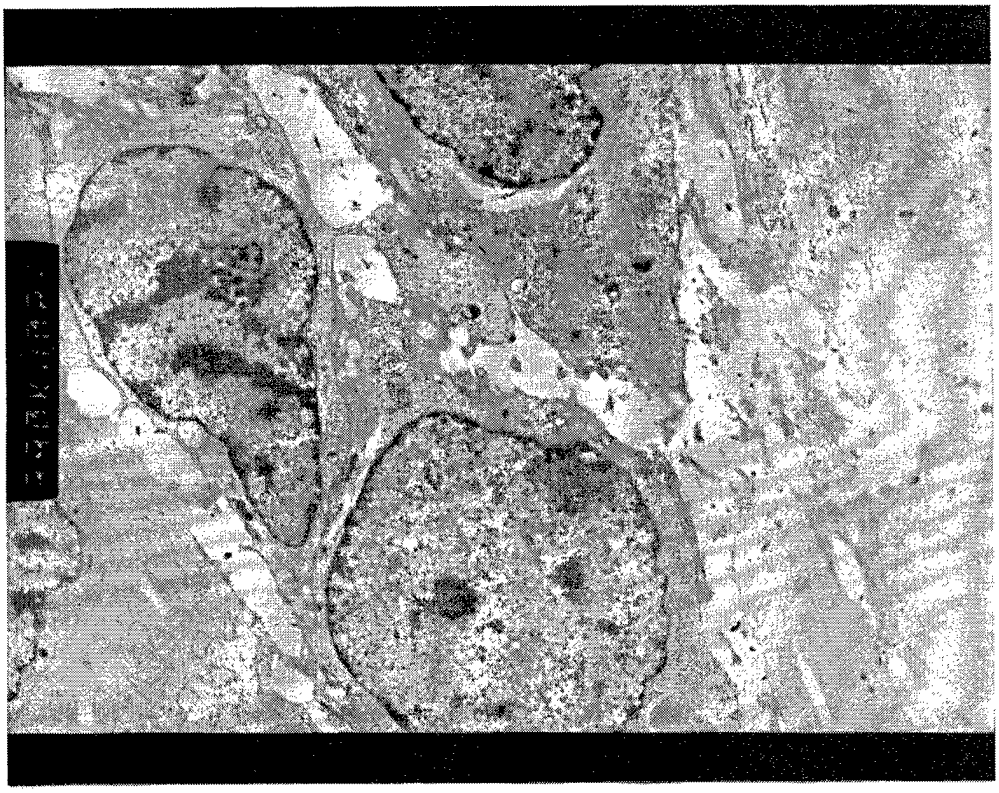

Figure 14. TEM: The tumor tissue in anode area immediately after 5 C of ECT.

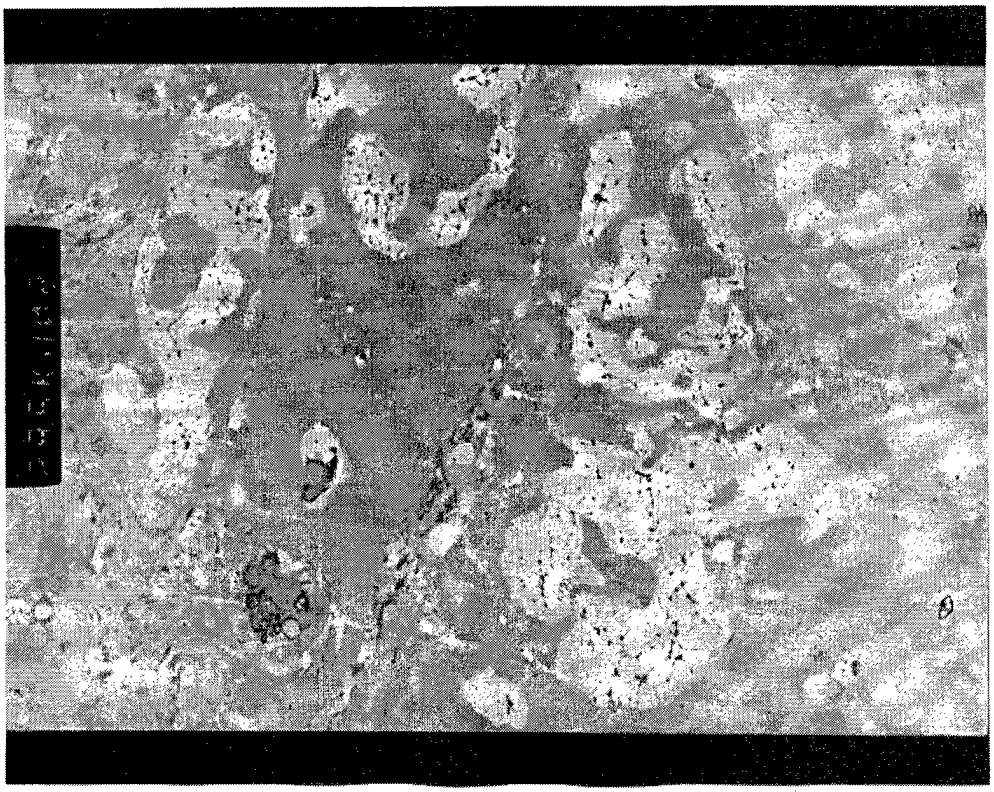

Figure 15. TEM: The tumor tissue in cathode area immediately after 5 C of ECT.

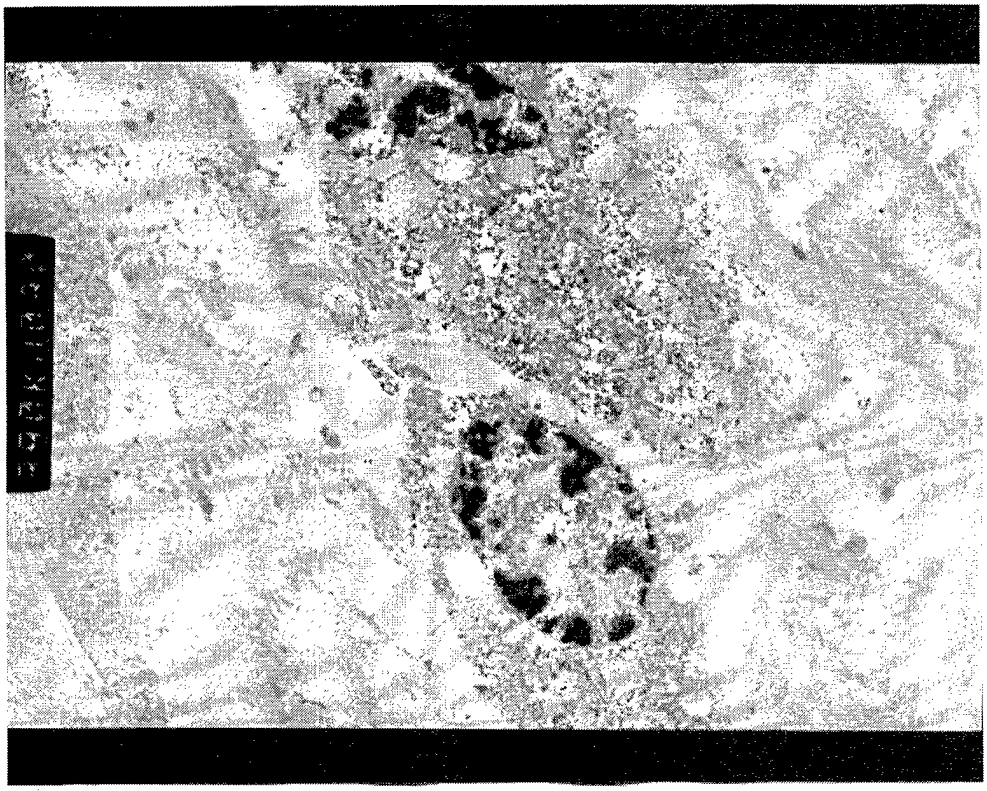

Figure 16. TEM: The tumor tissue in anode area immediately after 20 C of ECT.

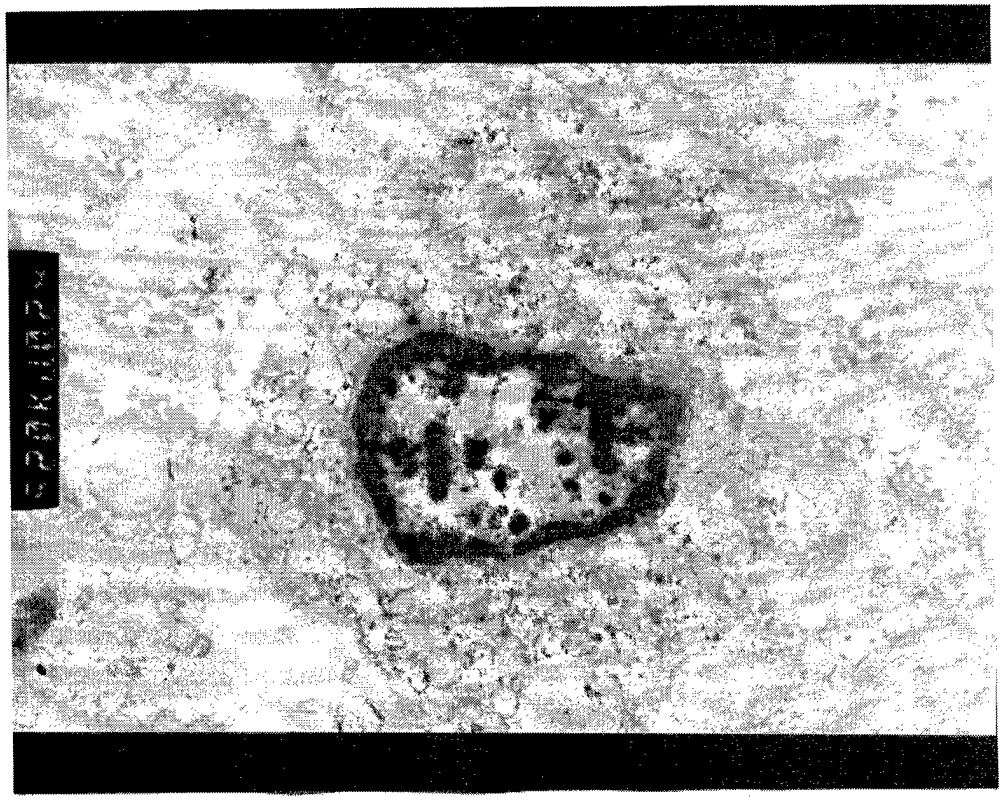

Figure 17. TEM: The tumor tissue in cathode area immediately after 20 C ECT.

DISCUSSION

Tumor cell selection:

As mentioned before, the references for ECT basic researches are slim even though ECT has been applied to treat many kinds of tumors clinically. When we submitted the proposal in 1996, the hypotheses of ECT for treating rat breast cancer were mainly based on our experimental ECT results on rat fibrosarcoma (9). According to our experience in creating the rat fibrosarcoma model, the tumor can grow to 4 × 4 × 4 cm without obvious necrosis inside the tumor, but the rat breast cancer is different. At first, we used MTLn 3 rat breast cancer cells to create a tumor model in Fisher 344 rats, as we described in the proposal. As the tumor grew to 2 × 2 × 2 cm, obvious necrosis and fluidifying appeared. This introduces complicating factors into the study and made it difficult to determine the effectiveness of ECT. When we used MTC rat breast cancer cells to create a tumor model, the same conditions happened as that of MTLn 3 cells. Last, we selected MTF 7 rat breast cancer cells (10) to create the tumor model. The tumor can grow to 2.5 × 2.5 × 2.5 cm without obvious necrosis and fluidifying; that is why we used MTF-7 cell line instead of MTLn 3 cell line in our study.

Effects of anodes and cathodes:

Since there is a lack of systemic and overall basic research, the method of ECT in clinic applications is not uniform. Some investigators insert both anodes and cathodes into the tumor tissue to induce the regression of tumor (6-8), while others insert only anodes into the tumor (1) because they believed that the anodes will draw tumor cells to it and cathodes will push tumor cells off since the surface of tumor cells carry negative charges (11-13). Our results indicated that both anodes and cathodes can destroy the tumor cell structures and induce necrosis of tumor tissue. Furthermore, both anodes and cathodes can induce degeneration and necrosis of muscles. Based on this observation, we infer that both the electrodes will destroy treated tissues. Therefore, inserting cathodes into normal tissues should be deterred because it will result in unnecessary damage of normal tissues. This complication may be totally avoided by inserting both anodes and cathodes into the tumor tissues.

ECT mechanisms:

It has been known that ECT involves electrolysis, electrophoresis and electroosmosis (14-17). Electrolysis and electrophoresis result in the decomposition of electrolytes. During electroosmosis, water moves from the anode to the cathode. This process dehydrated cells near the anode and hydrates cells near the cathode. That is why the tumor tissue shrank in the anode area and swelled in the cathode area. The water volume change within the cell disturbed the cell structure and function. In addition to the changes in water volume, Cl_2, which is formed at the anode due to Cl^- electron loss, may play a role in cell growth inhibition by its oxidizing effect. In addition, H_2 is formed at the cathode due to H^+ electron. These gas bubbles escapes along the electrodes during ECT. The mechanisms above explain the observed phenomena we observed. We believe that ECT induced cell death may involved much more complicated processes, such as pH alteration, ion concentration changes, and DNA single or double strand breaks. To better understand the effectiveness of ECT, further research in understanding the mechanisms must be conducted.

CONCLUSION

(1) The tissues around electrodes were necrosis after ECT, which indicated that ECT is effective for cellular disruption and death of the affected tissue;

(2) Both anode and cathode can damage and destroy cell structures, which indicated that the cathodes should not be inserted into the normal tissues;

(3) The effects of ECT are non-specific since it will destroy both tumor and normal tissues. Due to the non-specificity of its effect, all of the electrodes should be inserted into the tumor tissues.

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