Short-term observation of electric ablation with nanosecond pulsed electric field (nsPEF) on hepatic hilar area

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Research

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Abstract

Background: Treatment of liver malignancies located at the hepatic hilar area has long been recognized as a challenge for both surgeons and interventional radiologists. Traditional locoregional thermal ablative therapies like radiofrequency ablation (RFA) have natural deficiencies to achieve complete ablation. Nanosecond pulsed electric field (nsPEF) has emerged as a novel electric power based locoregional therapy. It has been reported to effectively ablate liver malignancies while its effect on tumors located in hepatic hilar areas still lacks well-controlled follow-up studies.

Methods: This pre-clinical study was conducted to evaluate the safety and feasibility of nsPEF ablation in the hepatic hilar area and to investigate its effect on liver vasculature systems. Two-needle electrodes of the nsPEF were placed around the hepatic hilar areas in the rabbit liver under ultrasonic guidance. During and after the procedure of nsPEF ablation, electrocardiographs (ECG) was used to monitor cardiovascular activities and ultrasonography was used to detect vascular changes. The blood samples and liver specimens were collected at pre-treatment and at 2 hours, 2 days, 7 days, 14 days and 28 days post-treatment.

Results: Histopathological studies showed the targeted portal area was ablated accurately without perivascular sparing. The major structures of the large hepatic veins and bile ducts near hilar areas were preserved well. Follow-up biochemical tests showed a transient and mild impairment of liver function. The results of myocardial enzymes and routine blood test proved nsPEF won't cause collateral damage on cardiac systems or increase potential infection risk. Ultrasonography and electrocardiographs found no massive hemorrhea and abnormal cardiac activities.

Conclusion: Our results demonstrate that nsPEF is safe and feasible in the ablation of large animal models. During the treatment, nsPEF would not disturb vital organ functions or cause irreversible complications. Furthermore, it can ablate the hepatic hilar area without damaging large hepatic vasculatures, which could be a promising method for non-thermal tumor ablation.

Background

Liver cancer was the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death worldwide(1). According to the latest guidelines, surgery is still the most important and effective approach to achieve long-term survival for liver cancer patients(2). However, some of the patients were already in advanced stage on first diagnosis and had lost the opportunity for surgery due to unresectable tumor size or liver cirrhosis. These patients have to resort to locoregional therapies like thermal ablations or transarterial chemotherapy to avoid invasive damage.

Thermal ablation is commonly used to treat the patients with poor liver function or tumor size less than 3 cm. However, traditional thermal ablations like radiofrequency (RFA) and microwave ablations (MWA) face major challenges during the treatment of liver tumors adjacent to large vessels. Firstly, flowing blood in the large vessels takes away thermal power during the ablation, which may lead to incomplete ablation

of tumor tissues, known as “heat-sink” effect(3, 4). Secondly, overheating causes damage on blood vessels and bile ducts which may lead to serious complications(5–7).

Benefited from updated pulsed power technology, a new electric local ablation technique known as nanosecond pulsed electric field (nsPEF) has emerged in cancer treatment. NsPEF characterizes for ultrashort pulse duration (nanosecond) and extremely high electric field intensity (higher than 10 kv/cm), which enables the electric current to breakthrough tumor tissue and achieve complete ablation(8–10). From the cellular level, nsPEF penetrates cellular membrane with high electric energy and produces irreversible nanopores, which leads to apoptosis of cancer cell(11, 12). Furthermore, some studies have proved that nsPEF enhances tumor antigen presentation of dendritic cells (DCs) and thus promotes adaptive immune response against cancers(13). In recent clinical trials, nsPEF also demonstrated encouraging therapeutic effects in human basal cell carcinoma (BCC) with few scars and reduced pain for patients(14).

Compared with conventional thermal ablative therapies, nsPEF ablate the tumor tissues based on high-intensity electric power instead of thermal energy (70–95℃)(15). This non-thermal characteristic of electric ablation makes it unaffected by the so-called “heat-sink” effects, theoretically. In the meantime, it should not cause uncontrolled thermal damage on vasculature systems. In consideration of the above features of nsPEF ablation, we speculated that nsPEF could be identified as the first option for locoregional ablation of the liver malignancies located around the large vessels or bile ducts, especially the hepatic hilar area. Therefore, we performed experiments to verify the safety, feasibility and efficacy of nsPEF ablation on hepatic tissues containing large vessels on non-tumor-bearing rabbit models.

We demonstrated for the first time that the standard nsPEF treatment with strict control of parameters could achieve uniform ablation in hepatic hilar areas without obvious side effects on large animals. Furthermore, the structure of large hepatic vasculatures including blood vessels and bile ducts in the ablation area was preserved well under nsPEF treatment, which proved its feasibility in the ablation of tumors around high-risk areas in the liver.

Results

NsPEF treatment won’t influence the blood flow of the large vessels

The ultrasound monitoring of one rabbit as a representative was demonstrated in Fig. 1. The distance between the two needles was about 10.5 mm (Fig. 1a). Massive hemorrhage was not found in the ablation area after puncture (Fig. 1b). There was a hypoecho area demonstrated in the ultrasonography 15 minutes post-treatment compared with pretreatment (Fig. 1c). The area was mostly consistent with the ablation region of the pathology. The color ultrasonic flow imaging showed a mild local circulation deficiency in the hepatic hilar area, and no thrombosis formation or massive circulation deficiency was found 15 minutes after the ablation (Fig. 1d).

NsPEF could preserve the structure of large vessels and achieve complete ablation

Figure 2 shows the gross anatomy of the liver specimens, including newly harvested tissues (Fig. 2a – 2e) and according formalin fixed tissues (Fig. 2f – 2j). It demonstrated the changes of the ablated area at different time points, including congestion or swelling (Fig. 2f – 2 g) – inflammation (Fig. 2g) – recovery (Fig. 2h) – fibrosis (Fig. 2i) – dissolution (Fig. 2j). A distinct border line between the ablation area and normal tissue was appeared on 14 days post-treatment (Fig. 2i). Figure 3 shows the pathologic changes of the ablated area. Extensive red blood cells infiltrated into the sinusoids in the ablation zone 2 hours post-treatment. Endothelial damage was hardly seen in the large blood vessels immediately after the ablation and the bile ducts was almost intact. On day 2, part of the endothelial layers of the large veins shed off but they preserved a complete structure. Massive inflammatory cells infiltrated into the ablated area. Some minor vessels were completely destructed, leaving a rough contour line of these vessels. The bile ducts in the ablated area showed mild inflammatory changes. Reendothelialization in the vessels occurred 7 days post-treatment and the large veins kept a complete construction. By 14 days, vascular congestion and infiltrating inflammatory cells had resolved in the ablated zone. Neovascularization appeared around the large vessels and in the margin of the ablated area. At 28 days post-treatment, the cell death in the ablated zone was completely replaced by regenerated hepatocytes and fiber matrix.

NsPEF ablation causes a repairable and slight damage to liver function

Figure 4 shows the changes of liver function during nsPEF treatment. The tested results included total protein (TP), albumin (Alb), globulin (Glb), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), direct bilirubin (DB), total bile acid (TBA). ALT and AST rose up transiently from 2 hours to 2 days post-treatment while TP and Alb demonstrated no obvious changes, indicated that liver function underwent temporary damage. There were no obvious changes of serum bilirubin (TB and DB), which suggest nsPEF didn't cause side effects on biliary systems. While TBA went through slight disturbance after the ablation and immediately returned to normal level. The ALP and Glb kept steady during the whole procedure. These results indicated that the damage caused by nsPEF on hepatic and biliary systems is repairable and slight.

NsPEF Ablation Won't Cause Myocardial Injury

Figure 5 shows that the myocardial enzymes including heart type creatine kinase (CK), creatine kinase isoenzyme (CK-MB), lactate dehydrogenase (LDH), and hydroxybutyrate dehydrogenase (HBDH) increased rapidly after the ablation and returned to a normal level at 2 days post-treatment. Meanwhile, the results of cardiac troponin I (cTnI) kept under 0.01 ng/ml before, during and after the whole procedure (Additional File 1: Table S1). These results showed that there was transient puncture damage on skeletal

muscles and hepatocytes instead of myocardial systems, and the damage was repairable and acceptable.

Peripheral blood tests showed no signs of infection after nsPEF ablation

Figure 6 shows the changes of routine blood test results including red blood cell count (RBC), white blood cell count (WBC), neutrophilic granulocyte percentage (NEUT), haemoglobin (Hgb), haematocrit (HCT), blood platelet count (PLT), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), mean lcorpuscular volume (MCV). There was no significant change of the above results compared with the untreated groups except for PLT. Hgb and RBC kept steady the whole procedure indicating no massive hemorrage. NEUT demonstrated no significant changes, which ruled out the nsPEF-treatment-related infections. PLT rose up at 2 days to 7 days post-treatment, suggesting a potential procoagulant status of the peripheral blood.

Discussion

Nanosecond pulsed electric field is an emerging locoregional electric ablative therapy that can be used to treat a variety of solid tumors, including melanoma(15), breast cancer(16), colon cancer(9), osteosarcoma(17), pancreatic cancer(18) and hepatocellular carcinoma(19). Non-thermal and ignorance-of-heatsink effect are two of the most significant features of the nsPEF ablation, which made it possible to be applied on the tumors located near the vital structures like porta hepatis. The operations on this area is recognized as complicated operations for surgeons and considered as relative contraindications for RFA(20, 21).

In this study, we demonstrated for the first time that nsPEF could achieve complete ablation on liver tissues and preserve the large vessels within ablation areas well. Conventional thermal ablations like RFA are based on excessive heat energy to cause necrosis of liver tissues, without discrimination of parenchymal hepatic tissues or matrix tissue like vascular fibers(22). However, the nsPEF relies on electric pulses to generate irreversible nanopores on the cell membrane and does no damage to other types of molecules, which explains why it has the potential to preserve cellular matrix of the large vessels. As the short-term and mid-term pathological follow up shows, the large vessels including veins and bile ducts in the ablated area kept a complete vascular structure and the perivascular tissues were ablated accurately with no sparing. It is confirmed that the large vessels were protected from the electric damage. Although,some of the large vessels occurred mild muscularis layers damage and endothelial loss the presence of the extracellular matrix greatly facilitated the reendothelialization process within 14 days. It could also explain the significant immune responses and rapid tissue healings observed in a series of nsPEF-treated researches(14, 16, 19, 23). Another interesting phenomenon is that compared with veins, bile ducts maintained better with a more complete structure and received less damage on the biliary

epithelial cells. According to previous studies about RFA, it is assumed that bile ducts are more sensitive to thermal damage and less vulnerable to electric stimulations(7, 24, 25).

The protective effects for vasculature systems, especially for bile ducts, could be validated by the blood test results. Serum bilirubin including TB and DB showed no obvious changes during the whole procedure, indicating that the nsPEF treatment didn't cause biliary complications like cholestasis secondary to bile duct strictures that resulted from the thermal damage, which was of high incidence after RFA(26). Besides, TBA underwent mild changes immediately after the treatment but then kept steady, suggesting that nsPEF may slightly disturb the metabolism of lipid. Notably, the number of blood platelet, namely PLT, went up at 2 days post-treatment and last to 7days, which raises the concern of thrombosis formation. Although the thrombosis was not found in histopathologic results and the PLT went down at 14 days post-treatment, the increasing trend of PLT indicated a procoagulant effect of nsPEF. Previous studies reported that thrombogenicity is one of the risk factors after thermal ablation, which lead to the administration of heparin(27). For the above reasons, the lesson and problem in thermal ablation worth further researches in nsPEF.

Aminotransferases (ALT and AST) of the liver function results underwent temporary increase, which was assumed to be released by the dead hepatic parenchymal cells. Their subsequent recovery confirmed the safety of nsPEF. Also, the results of TP and Alb proved that nsPEF doesn't impair the capacity of albumin synthesis for the liver. RBC and Hb from blood routine results kept at the same level, proved that no massive hemorrhage occurred during the treatment. In the meantime, the inflammatory results like the percentages of neutrophils didn't change significantly, which indicated the strict asepsis operation could effectively avoid infectious risks during ultrasound guided puncture process.

The effect of the electric ablation on the cardiovascular and skeletal muscle systems were investigated in this study. Previous studies proved the release of high voltage powers increases cell membrane permeability and opens a path for ion transport, which can induce cardiac arrythmias and defibrillation, leading to some unpredictable cardiac accidents(28–30). In addition, the electric stimulation on excitable tissues like motor nerves can cause the involuntary contraction of the muscles of the subjects which may impede the proceed of the treatment(31–33). However, in this study, we adopted the synchronization pulse generating system, which would automatically stop if the ECG detects abnormal heart activities, effectively protected cardiovascular muscles from the electric damage. We assumed that the increase of the myocardial enzymes of CK and CK-MB was caused by muscle puncture rather than myocardial injury and the level of Cardiac troponin I confirmed this assumption. CK-MB-related muscle damage and the mild increase of LDH and HBDH is affordable in liver cancer patients. In addition, the general anesthesia and insulated electrode needles prevented the contraction of skeletal muscles. These results proved that the appropriate operation of synchronization pulse generating system and general anesthesia in nsPEF treatment is effective and necessary to prevent the possible side effect on cardiac and skeletal muscles.

However, there are certain problems remained to be solved in the future experiments: (1) It is difficult to find the accompanying arteries of the large veins in hepatic hilar area under ultrasound guidance and

even harder to include the three kinds of vessels (hepatic veins, arteries and bile ducts) in the ablation zone at one time. Therefore, our research mainly focused on the effect of nsPEF ablation on large hepatic veins and bile ducts and some of the minor arteries. However, the effect of nsPEF on large arteries, which have faster speed of blood flow requires further verification. (2) The risk of thrombosis formation reflected by PLT should be evaluated in the long run. Such evaluation requires not only the reexamination of blood coagulation functions, but also the regular monitoring of the large vessels by ultrasound.

Conclusion

Overall, nsPEF is safe and feasible in the ablation of tissues at the hepatic hilar area and the minimally invasive characteristic of this electric ablation may expand its application on end-stage, poor-liver function HCC patients. Furthermore, it has a potentially protective effect on hepatic vasculature systems, which laid the evidence for it to ablate liver malignancies located near the porta hepatis. It is one of the newest techniques available for treating hard-to-reach tumors, offering another option for patients who have cancerous tumors that are close to blood vessels, ducts or nerves that may otherwise be damaged by heat-based ablation techniques, such as RFA, MWA and cryotherapy. Multiple center clinical trial investigations are ongoing to verify its clinical outcomes on patients and application prospects.

Material And Methods

Animals and anesthesia

This study was approved by the Animal Care and Use Committee of Zhejiang Academy of Medical Sciences. The anesthesia and treatment procedure were fully complied with the animal experimental guidelines. All animals received appropriate humane care from certificated professional staff. A total of 20 New Zealand White rabbits (2.2 kg ± 0.2 kg) were purchased and maintained by the Division of Experiment Animal Laboratory of Zhejiang Academy of Medical Sciences. General anesthesia was maintained with 1.5% – 2% isoflurane by mechanical ventilation during the procedure (Fig. 8c). The basic vital signs of each rabbits were monitored using an ECG machine and observed and documented by an experienced anesthetist (Fig. 8). Vital signs were stable during and after the whole treatment procedure. ECG didn't detect severe heart arrhythmia or other abnormal cardiac activities. Minor complications include subcutaneous hematoma and pneumothorax was not found during and after the nsPEF treatment.

The Nanosecond-pulsed Electric Field Treatment

All the nsPEF treatment procedures were performed with open laparotomy and the abdomen was closed after the ablation treatment (Fig. 8a). The pulse generator device was provided by Ruidi Biological Technology LTD (Hangzhou, Zhejiang, China) with two electrode needles (Fig. 8d). The effective tip length to generate electric field is 2 cm (Fig. 8b). The nsPEF treatment parameters were set as follows: each

pulse duration was 300 ns, 800 pulses were conducted in each treatment, pulse frequency was 2 Hz, electric field intensity was maintained at 25000 V/cm. Electrocardiograph was used to monitor the cardiac activities and to make sure the pulses were generated during the absolute myocardial refractory period to prevent heart arrhythmias. The parameters were set and adjusted according to our previous ablation experience on rabbits with liver cancer.

Ultrasound Guidance And Evaluation

Before treatment, ultrasonography was used to identify hepatic hilar area and guide the needle puncture (Fig. 8c).15 minutes after the ablation, width and length of the ablated area was measured by ultrasonography (Mylab Gamma, Esaote Group, Italy). The blood perfusion in and around the ablated area was monitored. All ultrasound procedure was performed by an experienced ultrasound doctor with more than 20 years practice experience.

Blood Biochemical Follow-up

Blood samples were collected at 2 hours before nsPEF treatment and at 2 hours, 2 days, 7 days, 14 days, 28 days post-treatment in order to dynamically observe the changes of liver function, myocardial enzyme assay and blood routine tests after the treatment (Fig. 7c). The results of blood tests 2 hours before treatment was set as the baselines. All the biochemical results were analyzed by an automatic analyzer (ARCHITECT i2000sr, Abbott, Illinois 60064, USA).

Histopathology Examination

The ablated livers were harvested at the above time points post-treatment. Liver vasculature was immediately flushed with 0.9% physiological saline solutions through the postcava for 5 minutes to maintain its physiological state. After the saline perfusion, the ablated liver tissue was dissected and fixed with formalin for haematoxylin and eosin (H&E) staining.

Statistical analysis

The statistical results of blood biochemistry including liver function, myocardial enzyme assay and routine blood tests were illustrated with column graphs using GraphPad Prism 8.0.1. The results of nsPEF-treated and untreated groups were compared and analyzed with unpaired t-test. The data was demonstrated as mean ± SD. The statistical significance of p-value was demonstrated with P* < 0.05, P** < 0.01, P *** < 0.001, ns: no significance.

Abbreviations

nsPEF:Nanosecond pulsed electric field; HCC:Hepatocellular carcinoma; IRE:Irreversible electroporation; RFA:Radiofrequency ablation; ECG:Electrocardiograph; TP:total protein; Alb:albumin; Glb:globulin;

ALT:alanine aminotransferase; AST:aspartate aminotransferase; ALP:alkaline phosphatase; TB:total bilirubin; DB:direct bilirubin; TBA:total bile acid; CK:creatine kinase; CK-MB:creatine kinase isoenzyme; LDH:lactate dehydrogenase; HBDH:hydroxybutyrate dehydrogenase; RBC:red blood cell count; WBC:white blood cell count; NEUT:neutrophilic granulocyte percentage; Hgb:haemoglobin; HCT:haematocrit; PLT:blood platelet count; MCH:mean corpuscular haemoglobin; MCHC:mean corpuscular haemoglobin concentration; MCV:mean lcorpuscular volume; MWA:microwave ablation.

Declarations

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Authors' contributions

SSZ and ZW conceived and designed the experiments. JHL and JJQ performed the experiments and drafted the manuscript. SYZ, XHC, SYY and LZ participated in the experiments and analyzed the data. SSZ and WZ oversaw of all aspects of the study. All authors read and approved the manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Animal Care and Use Committee of Zhejiang University. The methods were carried out in accordance with the approved guidelines. All animals received appropriate humane care from certificated professional staff.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Supplementary File Legend

Additional file 1: Table S1. Results of Cardiac Troponin I (ng/ml).

Figures

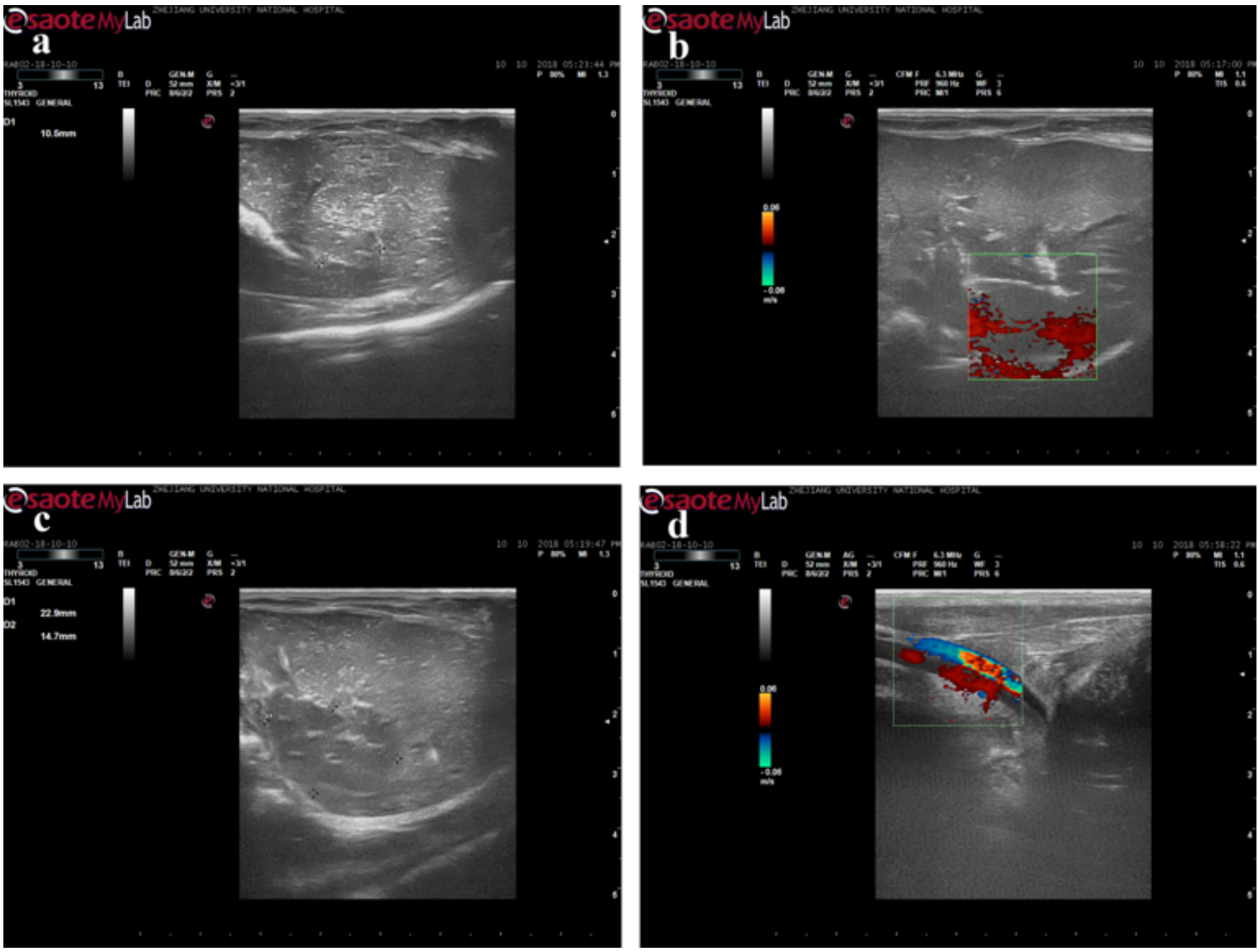

Figure 1

ultrasonography of one rabbit during the nsPEF treatment. (a) Distance between the two needles under ultrasound guidance was 10.5mm. (b) Ultrasonography confirmed there was no puncture related vessel damage. (c) The ultrasonography image demonstrated a 14.7 mm * 22.9 mm hypo echoic area 15 minutes after the ablation. (d) The blood flow situation was observed after the treatment.

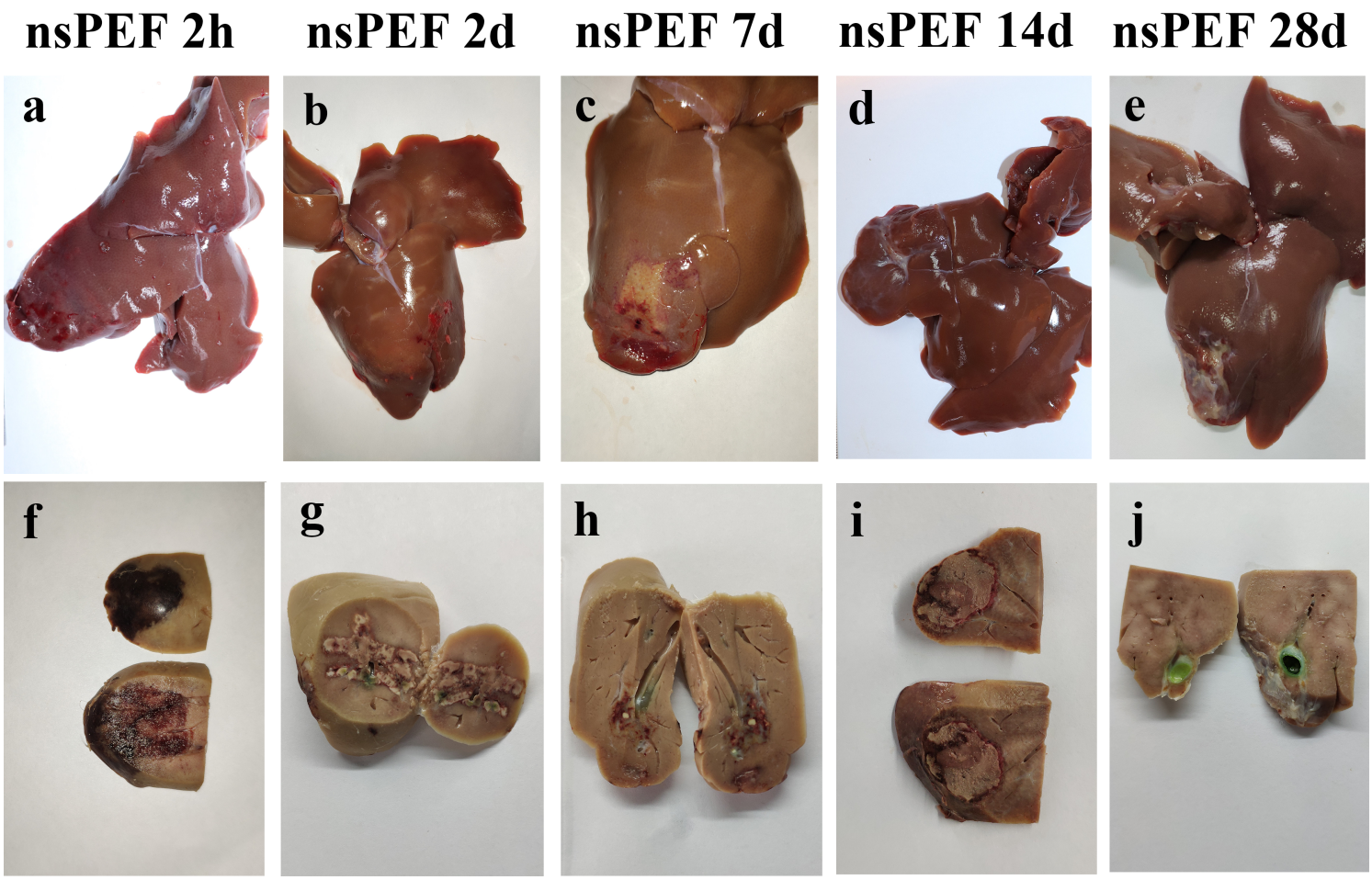

Figure 2

Pathological specimen of the nsPEF-treated liver tissues. The upper and lower groups of the figures represent the unfixed and formalin fixed liver tissues of 2 hours, 2 days, 7 days, 14 days and 28 days post-treatment, respectively. At 14 days post-treatment, there is a clear demarcation line between the treated area and normal liver tissue. The congestion and swelling were obvious in the treated area but partly resolved at around 7 days post-treatment. At 14 days post-treatment, some of the treated area became fibrosis and gradually wrapped with omentum. By day 28, the congestion was almost gone and the ablated area shows conglutination with the peritoneum. The vessels and biliary systems seen traversing the area appeared intact.

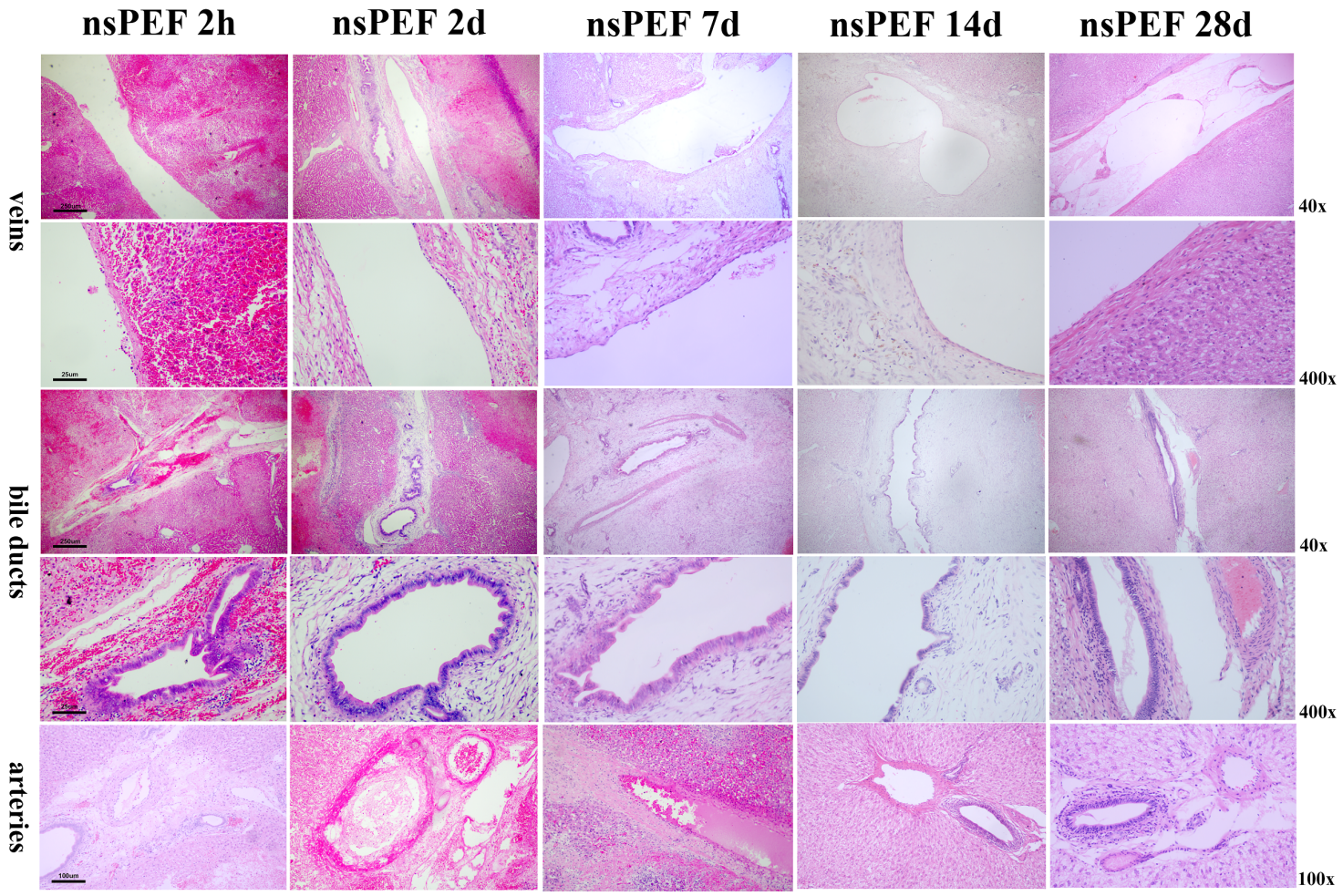

Figure 3

Haematoxylin and eosin (H&E) staining sections. Figure 3 shows the different hepatic vasculatures after the ablation in time order, including hepatic veins and bile ducts. Scale bars are in the lower left corner of each first pictures. At 2 hours post treatment, the hepatic sinusoids were infiltrated with massive amount of red blood cells. Most of the large vessels kept a complete structure and the endothelial layer kept intact, while part of the endothelial layers shed off from the vessel wall. The bile ducts in the ablated area showed no acute damage. By 2 days, some of the micro veins in the ablated area were destructed and the large vessels, though structurally preserved, demonstrated partial endothelial loss, neutrophil infiltration, and mild vasculitis. The bile ducts in the ablation zone showed mild signs of edema but no necrosis. Extensive red blood cells, granulocytes and other inflammatory cells infiltrated into the sinusoids. At the 7th day, the inflammation resolved in most of the area and the damaged endothelium of the large vessels began the process of reendothelialization. Some of the neovascular appeared around the large vessels and the margin of the ablated area. From the 14 days to 28 days post-treatment, the dead hepatocytes were gradually replaced by fibroblast tissues. Hepatocellular regeneration appeared in most of the ablated area.

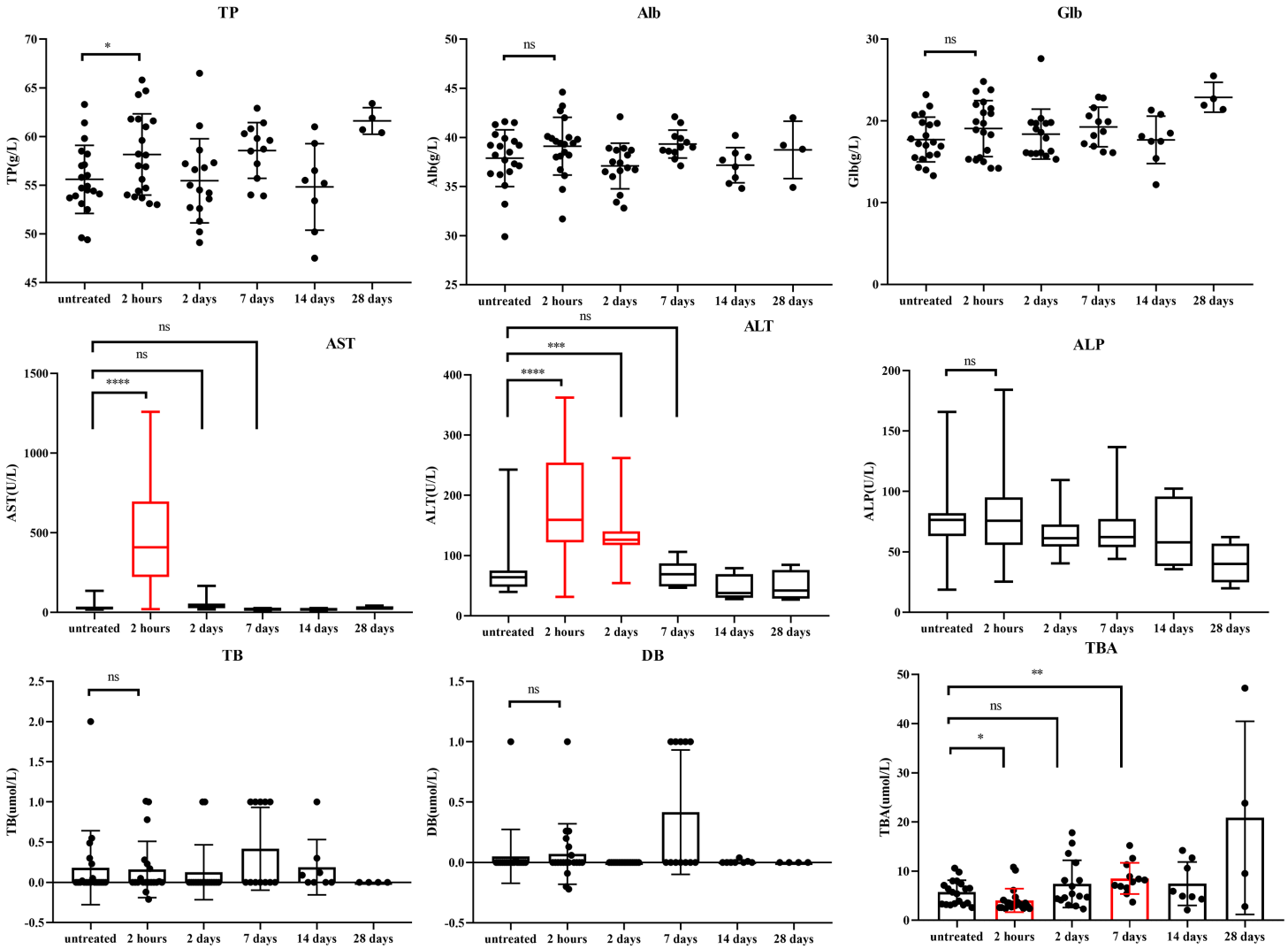

Figure 4

Liver function results. Liver function of each rabbit post treatment was compared with their untreated counterparts in time order, which illustrated a mild disturbance of liver functions post-treatment and subsequent recovery. The tested liver function results included total protein (TP), albumin (Alb), globulin (Glb), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), direct bilirubin (DB), total bile acid (TBA). The blood samples were collected pretreatment and at 2 hours, 2 days, 7 days, 14 days, 28 days post-treatment. Data were presented as mean±sd. P* < 0.05, P** < 0.01, P *** < 0.001, ns: no significance.

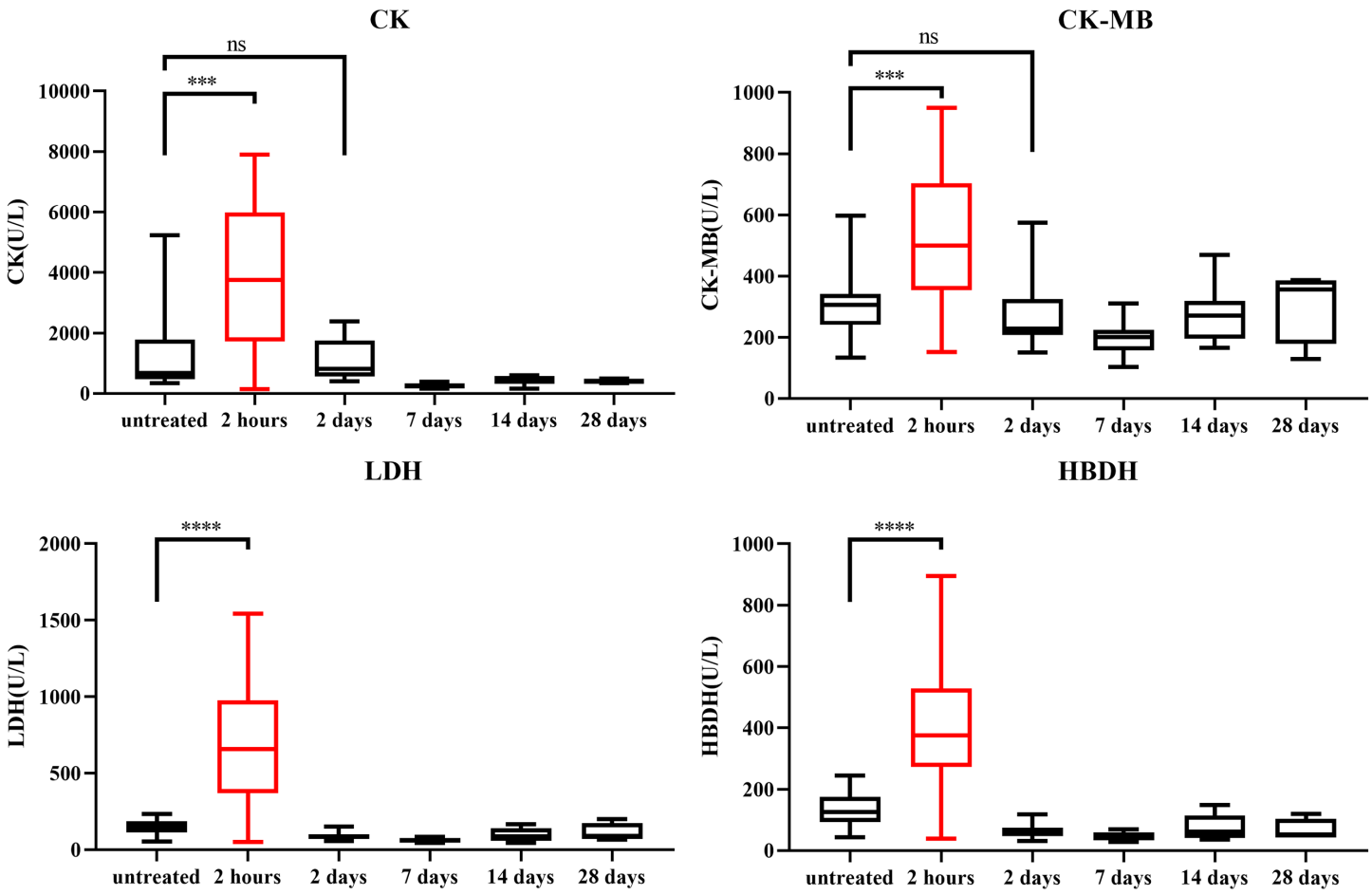

Figure 5

Myocardial enzymogram follow-up. Myocardial enzyme assay of each rabbit post treatment was compared with their untreated counterparts in time order, which demonstrated transient stimulation-induced damage of myocardial enzyme assays and self-recovery process without sequelae. Myocardial enzyme assay indexes included heart type creatine kinase (CK), creatine kinase isoenzyme (CK-MB), lactate dehydrogenase (LDH), and hydroxybutyrate dehydrogenase (HBDH). The blood samples were collected at pretreatment and 2 hours, 2 days, 7 days, 14 days, 28 days post-treatment. Data were presented as mean±sd. P* < 0.05, P** < 0.01, P *** < 0.001, ns: no significance.

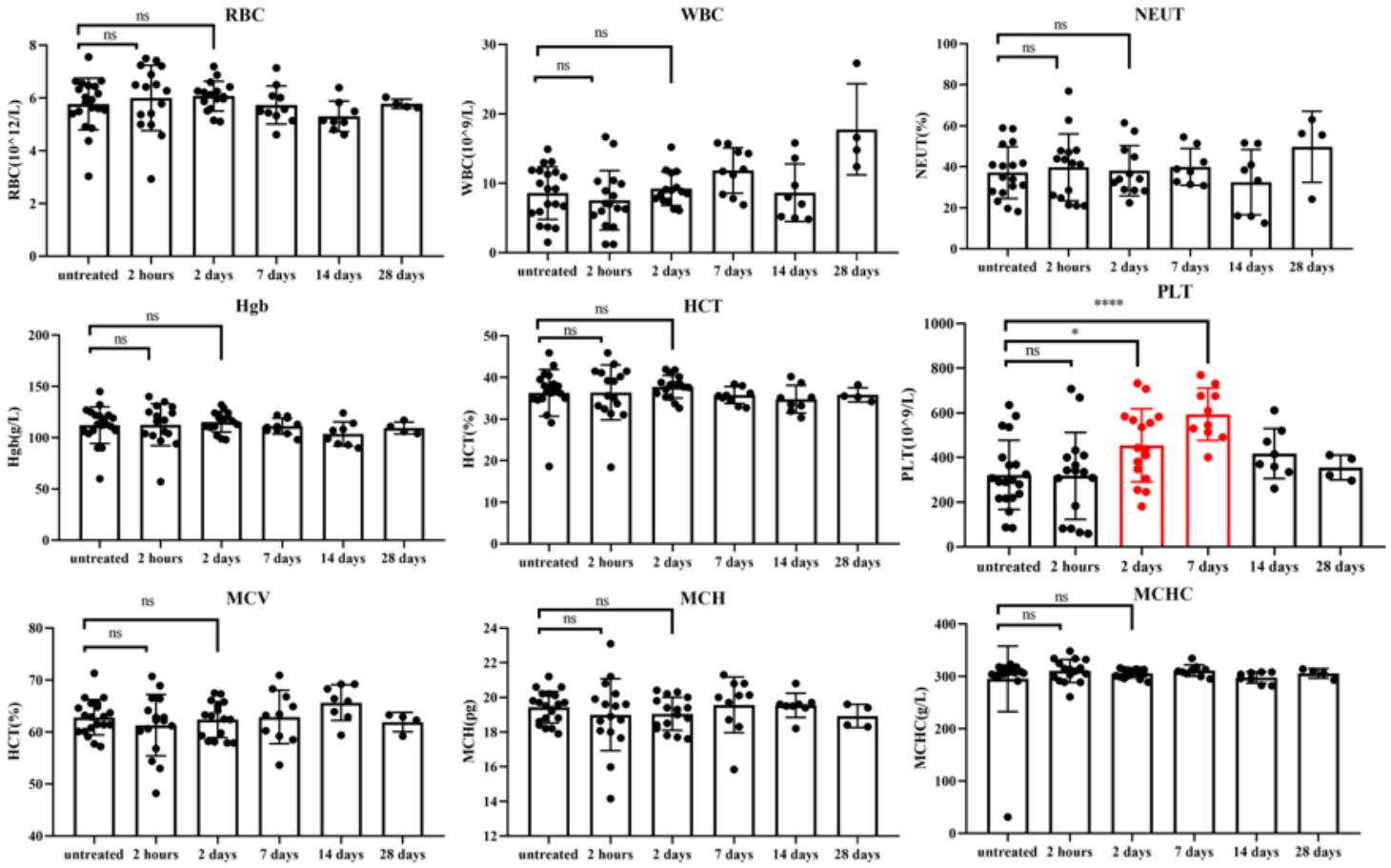

Figure 6

Routine blood test follow-up. Routine blood test of each rabbit post treatment was compared with their untreated counterparts in time order, which showed no obvious changes and indicated controlled risk of infections during the treatment. Routine blood test results included red blood cell count (RBC), white blood cell count (WBC), neutrophilic granulocyte percentage (NEUT), haemoglobin (Hb), haematocrit (HCT), blood platelet count (PLT), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV). The blood samples were collected at pretreatment and 2 hours, 2 days, 7 days, 14 days, 28 days post-treatment. Data were presented as mean±sd. P* < 0.05, P** < 0.01, P *** < 0.001, ns: no significance.

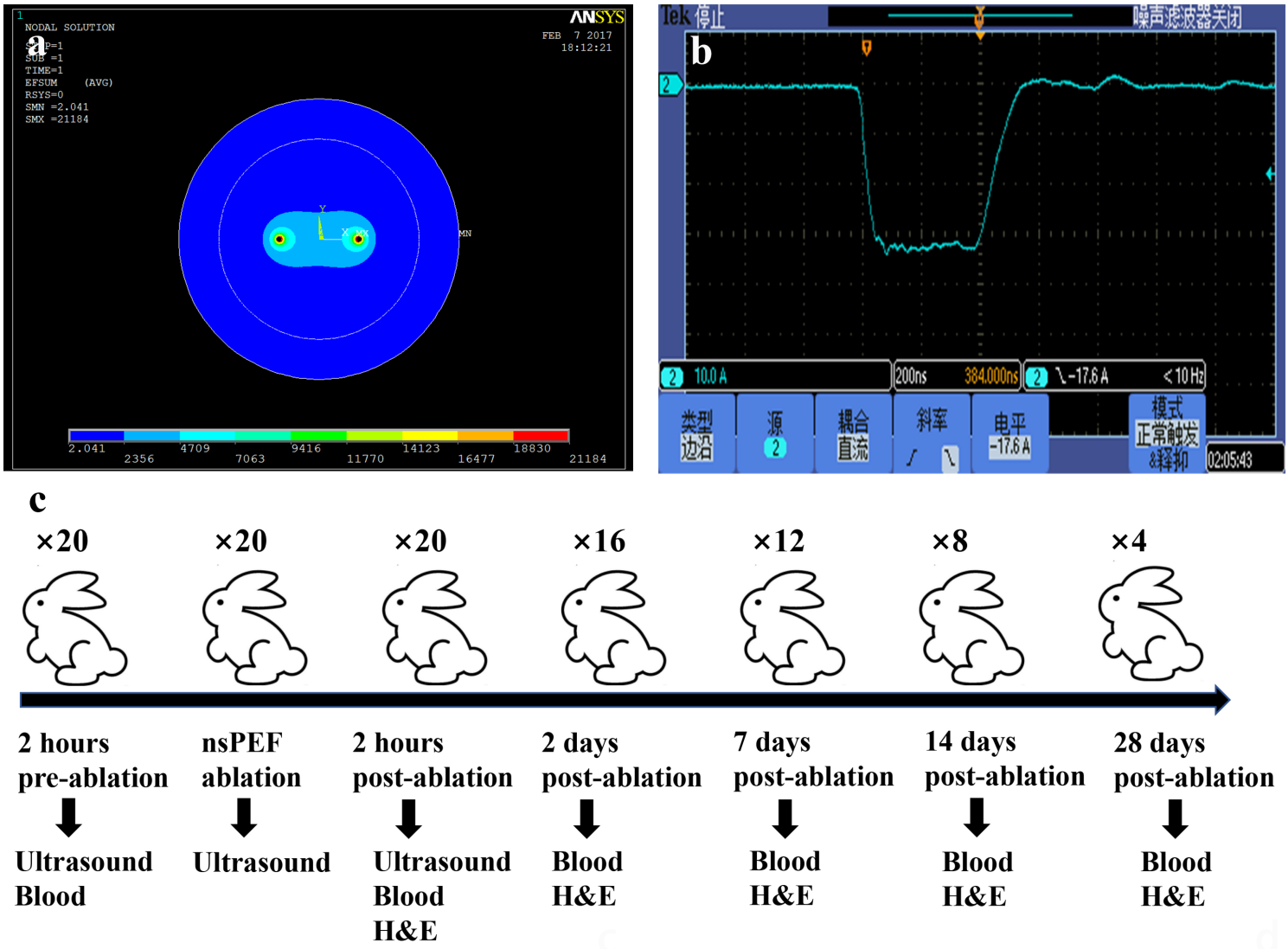

Figure 7

Schematic diagram of nsPEF and the animal experiment design. (a) The ablation area and its according temperature was pre-calculated by software simulation according to the distance between the two electrodes. (b) The pulse shape was monitored during the procedure. (c) A total of 20 rabbits accepted the nsPEF ablation. Every four rabbits were euthanized at 2 hours, 2 days, 7 days, 14 days and 28 days post-treatment. The blood samples and liver tissues were obtained at each of the time points.

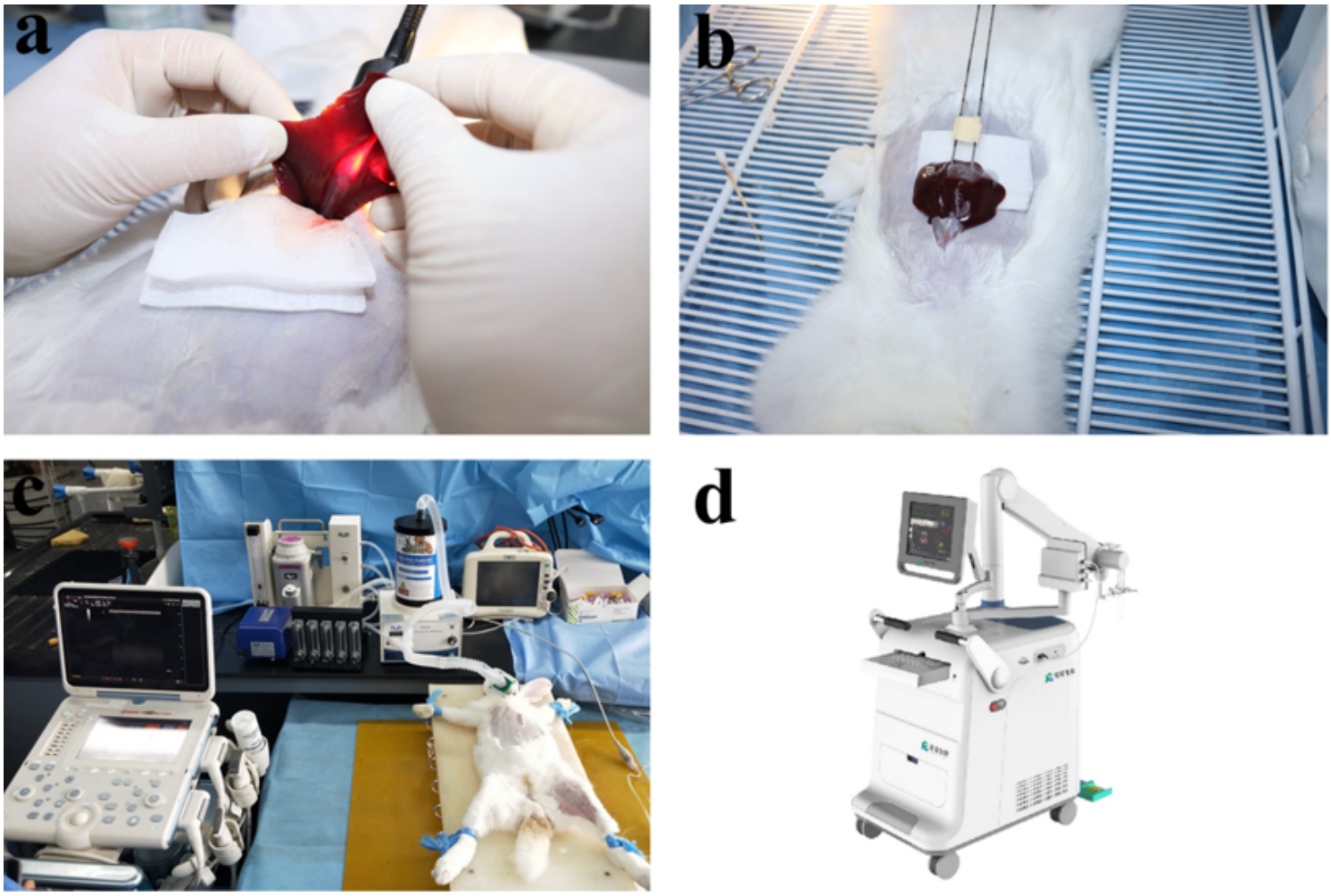

Figure 8

Nanosecond pulsed electric field treatment settings. (a) The treatment was performed by open laparotomy to directly identify the location of porta hepatis where bile ducts and large vessels converged. (b) The two-needle electrode was inserted into the identified location with the tip depth of about 2 cm. (c) The general anesthesia was maintained with mechanical ventilation. (d) The prototype of nsPEF used in this experiment.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.xlsx