Video Article

# Assessment of Plasma Coagulation on Liver Tissue in a Large Animal Model *In Vivo*

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## Abstract

Plasma coagulation as a form of electrocautery is used in liver surgery for decades to seal the large liver cut surface after major hepatectomy to prevent hemorrhages at a later stage. The exact effects of plasma coagulation on liver tissue are only poorly examined. In our porcine model, the coagulation effects can be examined close to the clinical application. A combined laser Doppler flowmeter and spectrophotometer documents microcirculation changes during coagulation at 8 mm tissue depth noninvasively, providing quantifiable information about hemostasis beyond the subjective clinical impression. The temperature at coagulation site is assessed with an infrared thermometer prior and post coagulation and with a thermographic camera during coagulation, a measurement of the gas beam temperature is not possible due to the upper threshold of the devices. The depth of coagulation is measured microscopically on hematoxylin/eosin stained sections after calibration with an object micrometer and gives an exact information about the power setting-coagulation depth-relation. The sealing effect is examined on the bile ducts as it is not possible for a plasma coagulator to seal larger blood vessels. Burst pressure experiments are carried out on explanted organs to rule out blood pressure related effects.

## Video Link

The video component of this article can be found at https://www.jove.com/video/57355/

## Introduction

Argon plasma coagulation (APC) is a widely used instrument in abdominal surgery for more than three decades[1,2]. It is a standard technique for the achievement of secondary hemostasis after major hepatectomy by sealing the liver cut surface to prevent later hemorrhages[3]. Plasma coagulation is a specialized form of radiofrequency electrocautery, which delivers the electrical energy through an arc of ionized gas. Providing monopolar electrothermal hemostasis, this noncontact technique has the advantage of preventing the electrode to stick to the tissue[4]. The ionized gas beam is automatically directed to the area of the lowest electrical resistance and is turned away when resistance rises due to desiccation to other areas not yet desiccated. This produces a uniform limited depth of coagulation[5,6]. Factors influencing the coagulation effect are the activation time, the power setting of the coagulation device and the distance from the probe to the tissue. Helium is another carrier gas, which can be used for plasma coagulation[7]. Recent clinical studies concentrated on clinical outcomes rather than histological and functional findings[3,8,9], while experimental studies focused on *in vitro* investigations[10] or experiments on isolated perfused organs[11].

The underlying protocol allows the study of the effects of plasma coagulation in a large animal model close to the clinical application using standard human equipment on pigs: Microcirculation is assessed noninvasively by a laser Doppler flowmeter and spectrophotometer, which is a standard clinical tool for this indication[12,13]. Temperature changes during coagulation are monitored with an infrared thermometer and a thermographic camera. The depth of coagulation is measured on histological hematoxylin/eosin stained sections after harvesting of tissue samples. For the comparison with other means for secondary hemostasis, burst pressure experiments are performed. In contrast to previously described techniques[14], these are conducted on explanted organs to exclude blood pressure related effects. In addition to the described investigations on the local effects of plasma coagulation, standard blood tests can also be undertaken in the porcine model.

## Protocol

Rules governed by German legislation for animal studies as well as Principles of Laboratory Animal Care (National Institutes of Health publication ed. 8, 2011) were followed. Official permission is granted from the governmental animal care office (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, Recklinghausen, Germany).

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## 1. Animals

1. Use female German landrace pigs (weighing 25-30 kg) housed in open cages.
2. Use 5 animals per group (argon and helium).
3. Allow the animals to acclimatize to the surroundings for at least one week before the experiments. Fast animals for 24 h prior to surgery with free access to water.

## 2. Anesthesia

1. Premedicate the animals with an intramuscular injection of ketamine (15 mg/kg body weight [BW]), xylazine (10 mg/kg BW), and atropine (0.1 mg/kg BW) 10 min before induction of anesthesia.
2. Peripheral venous access is established by placement of a 22-gauge cannula into an ear vein.
3. Induce general anesthesia by i.v. injection of propofol 2 mg/kg body weight.
4. Place the animal in the supine position and perform a longitudinal skin incision in the jugular groove over a length of 2 cm. Locate vein through the blunt preparation of the subcutaneous tissue. Insert cannula, then Seldinger wire.
5. Retract cannula and insert 14 Fr. catheter over the guide wire. Retract guide wire. Connect catheter to the extension and fixate the catheter by a strap or a suture.
6. Pull out the tongue and insert straight laryngoscope. Use the tip of the laryngoscope to pull down the epiglottis. Insert the tube through the vocal cords. Place cuff under glottis and inflate.
7. Ventilate with 40% oxygen at 20-26 breaths/min and a tidal volume of 10 ml/kg to keep the end-tidal partial carbon dioxide tension between 36 and 42 mm Hg.
8. Maintain anesthesia with isoflurane at a concentration of 1-1.5% and fentanyl at a concentration of 3-4 µg/kg/h.
9. Supply Ringer's lactate solution at an initial rate of 4 mL/kg/h, and increase after laparotomy to a constant infusion rate of 8 mL/kg/h.

## 3. Surgery and Plasma Coagulation

1. Place the animal in a supine position on a standard surgical table.
2. Disinfect skin by applying a standard surgical disinfectant (2-Propanol 45 g /100 g, 1-Propanol 10 g / 100g, Biphenyl-2-ol 0.2 g / 100 g) with a surgical swab for 3 times.
3. Perform a wide midline laparotomy from the xiphoid process to the pubis with a scalpel and install surgical retractors.
4. Switch on the plasma coagulation device, open argon or helium gas bottle, depending on the used carrier gas. Adjust gas flow to 3 L/min. Select coagulation device output power as desired.
   NOTE: Both noble gases, Argon or Helium, can be used for plasma coagulation. Coagulation effects are comparable. See reference[7] for details.
5. Perform plasma coagulation on the left liver lobe as previously described[7]. Use a titanium mold (square aperture 1 x 1 $cm^2$) to standardize the coagulation zone. Coagulate for 5 s with a probe distance of 1 cm. The coagulations with different power settings can be performed side by side with a short distance between the coagulations of 5 mm (**Figure 1**).
6. For harvesting of the liver, divide all ligamentous connections to the liver. Isolate and divide the hepatic pedicle above the superior duodenal flexure leaving long portions of portal vein and common bile duct. Divide the caval vein above and below the liver and retrieve the organ.
7. After harvesting the liver, the pigs were euthanized by i.v. administration of 0.16 g/kg BW pentobarbital.
8. For the burst pressure experiments, resect half of the left medial liver lobe with sharp scissors. Plasma-coagulate the cut surface (100W output power) or seal the cut surface with fibrin sealant (**Figure 2**).

## 4. Microcirculation Measurement

NOTE: Laser Doppler spectroscopy can determine blood flow in tissue through measuring the Doppler shift caused by the movement of erythrocytes. The Laser signal correlates with the number of moving erythrocytes. Laser Doppler spectroscopy is in clinical use (e.g. transplant medicine) and has been validated multiple times[15].

1. Switch on the laser Doppler flowmeter and spectrophotometer. Use a flat probe.
2. Take baseline measurements for flow and velocity. Save or note the values.
3. Perform coagulation as described under 3.5.
4. Place the flat probe on coagulation sites and measure flow and velocity. Again, save or note values.
5. Repeat for all power settings of the coagulator device.

## 5. Temperature Measurement

1. Switch the system on (thermographic camera, notebook, and infrared thermometer) and let it run for at least 1 h before performing measurements.
2. Adjust focus and view frame on the thermographic camera on the coagulation site. Infrared sequences can be detected with the spatial resolution of 1024 x 768 pixels with a temperature resolution bigger than 20 mK. Take into account, that the region of coagulation and the surrounding tissue — affected by heat transfer — is located in the middle of the view.
   NOTE: It should include as many pixels of the frame as possible for an optimal spatial resolution.
3. Record coagulation process with the plasma coagulator on the liver surface with the thermographic camera over a 2-min period.
4. Analyze image sequences with the thermography analysis software: Define regions of interest.
   NOTE: Software calculates the course of corresponding mean temperature over time.

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## 6. Coagulation Depth Measurement

1. Harvest the left medial liver lobe with sharp scissors.
2. Excise the coagulation sites with 1 cm thickness. Cut into 3 mm thick longitudinal segments for further processing.
3. Fix tissue samples at 4 ° C overnight with neutral 10% buffered formalin. Heat paraffin 2 °C over the melting point and embed slices. Process overnight.
4. Perform Hematoxylin/Eosin staining.
    1. Deparaffinize and hydrate the tissue by subsequently dipping in 2x Xylene, 100% ethanol (EtOH), 95% EtOH, 70% EtOH, deionized $H_2O$ for 2 min each.
    2. Stain the tissue sample with Meyer's Hematoxylin solution for 3 min.
    3. Now rinse in tap water for 5 min.
    4. Stain the tissue with Eosin solution for 3 min.
    5. Rinse in 2x EtOH 95% and then Xylene for 3 min each. Mount with the standard mounting medium.
5. Switch the system on (microscope connected camera, imaging software). View all sections with 40X magnification.
6. Take an image of an object micrometer at a magnification of 40X. Press Recalibrate button in the Objectives window. Select manual calibration. Draw a line on the micrometer image of 100 µm. Enter 0,1 mm in the dialog box and press OK.
7. Select Length in the View>Analysis Controls>Annotations and Measurements window. Measure from the liver surface to the coagulation margin with the mouse. Export or note the result. Repeat measurement on another location on the same slide.
   NOTE: Coagulation depth can easily be differentiated from the normal liver tissue by the sharp margin between the normal hepatocyte cords and the zone of necrosis with shrunken cytoplasm, pyknotic nuclei, and hemorrhage zones.
8. Calculate mean out of two measurements.

## 7. Burst Pressure Measurement

1. Switch the system on (automatic pumps, pressure meter). Prepare the liver samples according to the step 3.7.
   NOTE: Use two parallel pumps connected via a 3-way-stopcock. The maximal pressure of 1,500 mm Hg cannot be obtained with a single pump.
2. Isolate portal vein, common hepatic artery and bile duct with scissors in the haptic pedicle. Clamp portal vein with an overholt forceps and ligate with a monofil suture 4-0. Clamp common hepatic artery an overholt forceps and ligate with a monofil suture 4-0.
3. Insert Ch-16 catheter into the common bile duct and ligate with a 2-0 silk suture. Connect the catheter to the automatic pumps, install 3-way stopcock with pressure meter (**Figure 3**).
4. Fill the perfusion syringe with saline.
5. Start automatic pumps with a delivery rate of 99 mL/h.
6. Monitor liver cut surface and pressure meter for leakage and record burst pressure.
   NOTE: For easier recognition of leakage, patent blue can be added to saline (2 mL patent blue + 18 mL saline). It is easier to observe burst pressure by noticing the time of loss of pressure on the pressure meter.

## Representative Results

**Microcirculation:** Utilizing the diagnostic device for hemostasis following plasma coagulation can be demonstrated by microcirculation changes. Capillary blood flow (displayed as arbitrary units (AU)) decreases from a baseline value of 142.7 ± 76.08 AU to 57.78 ± 49.57 AU at 25 W device output power, to 48.5 ± 7.26 AU at 50 W and to 5.04 ± 1.31 AU at 100 W (**Figure 4**).

**Temperature:** Temperature at the coagulation sites was measured with a thermographic camera (**Figure 5**). The only insignificant temperature changes were documented with an infrared thermometer. It showed a baseline temperature of 32.42 ± 2.27 ° C. After coagulation with 25 W, the temperature was 33.33 ± 1.81 °C. Coagulation with a 50 W laser yielded a temperature of 31.17 ± 2.13 ° C. After coagulation with the maximal power setting of 100 W, the temperature was mostly unchanged with 30.17 ± 3.19 ° C (**Figure 6**).

**Coagulation depth:** Plasma coagulation creates a superficial zone of necrosis with can be easily distinguished from the normal liver parenchyma (**Figure 7**). The depth of necrosis can be measured at multiple sections and shows a not completely linear increase with rising power levels of the plasma coagulator. Following helium plasma coagulation, the coagulation depth is 230.2 ± 57.83µm at 25W, 314.6 ± 87.39 µm at 50 W, 292.2 ± 45.65 µm at 75W and 412.9 ± 160.9 µm at 100 W device output power (**Figure 8**). The output power of the device can be chosen freely and chose a positive correlation with coagulation depth[7].

**Burst pressure:** Burst pressure measurements carried out on the cut surface of the explanted left medial liver lobe shows no difference after helium (1254±578.7 mmHg) or argon (1003 ± 554.4 mmHg) plasma coagulation (**Figure 9**). Burst pressures are lower compared to fibrin sealants[7] but seem appropriate for clinical use.

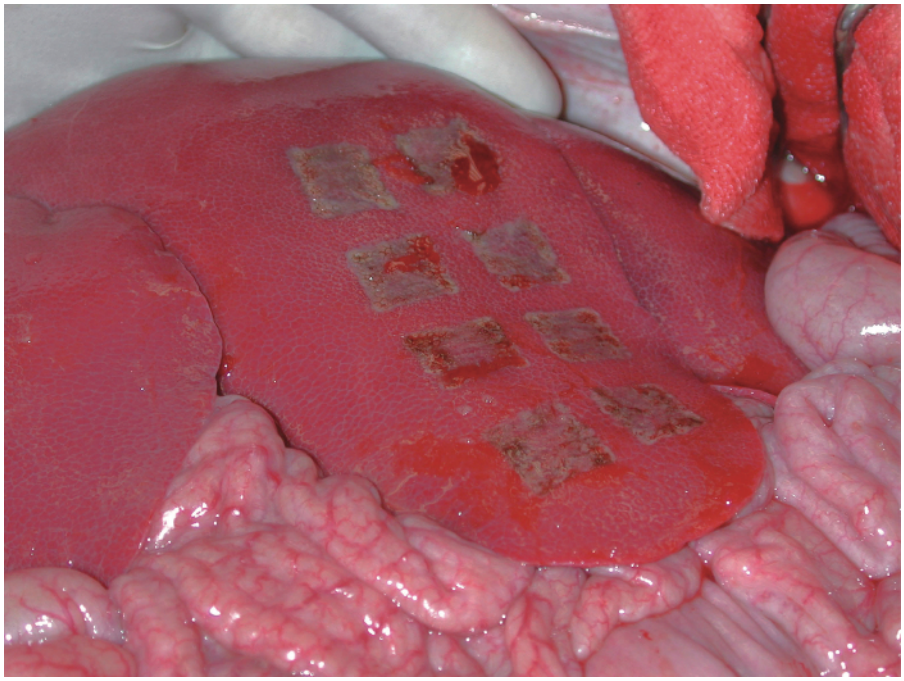

**Figure 1: Left medial liver lobe after argon plasma coagulation.** Eight coagulation sites on the left medial liver lobe (from top left to bottom right: 10 W, 15 W, 20 W, 25 W, 30 W, 50 W, 75 W, 100 W). The extent of coagulation standardized with the mold. Please click here to view a larger version of this figure.

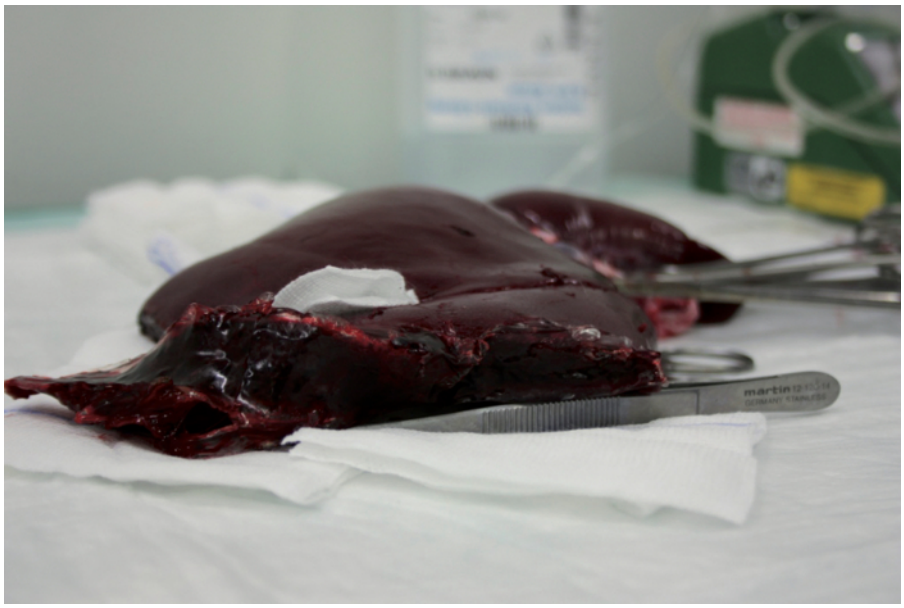

**Figure 2: Preparation of liver graft for burst pressure measurements.** Half of the liver lobe is resected, and liver cut surface is sealed with fibrin sealant. Please click here to view a larger version of this figure.


**Figure 3: Equipment for burst pressure measurements.** Automatic pump (syringe filled with saline) and pressure meter connected via a 3-way-stopcock. Please click here to view a larger version of this figure.

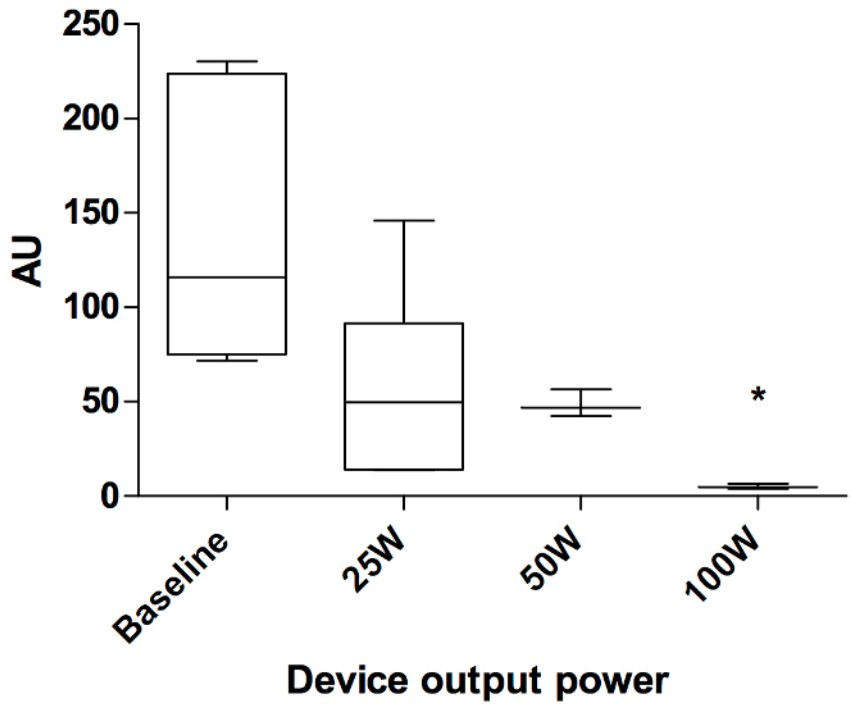


**Figure 4: Microcirculation changes.** Changes in blood flow (displayed as arbitrary units) before and after argon plasma coagulation at 25 W, 50 W and 100 W device output power (n=3-6). * = *P*<0.05, 1-way ANOVA. Please click here to view a larger version of this figure.

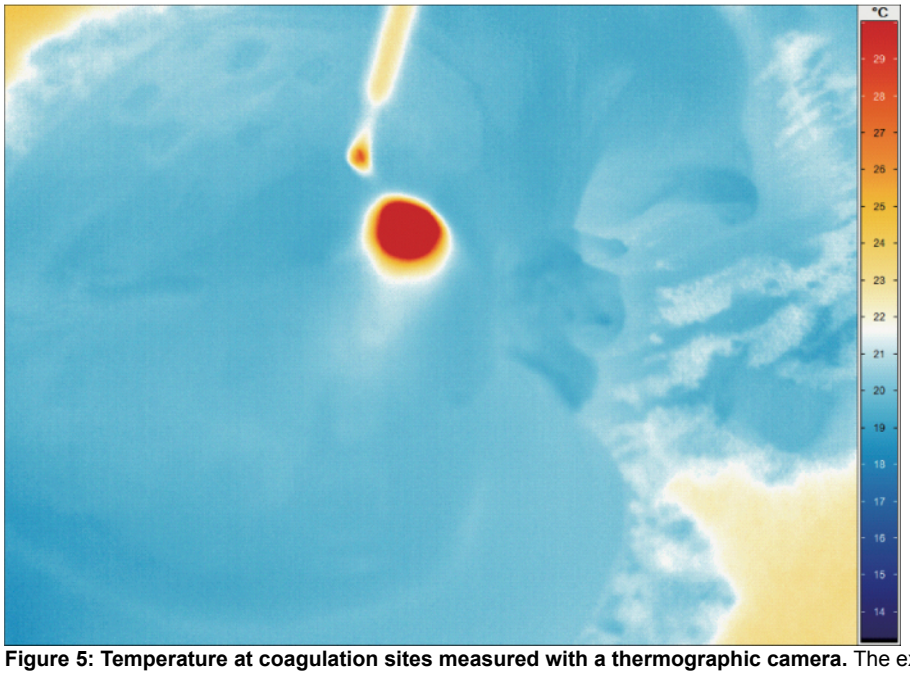


**Figure 5: Temperature at coagulation sites measured with a thermographic camera.** The exemplary picture with a thermographic camera during helium plasma coagulation with 40W device output power. Please click here to view a larger version of this figure.

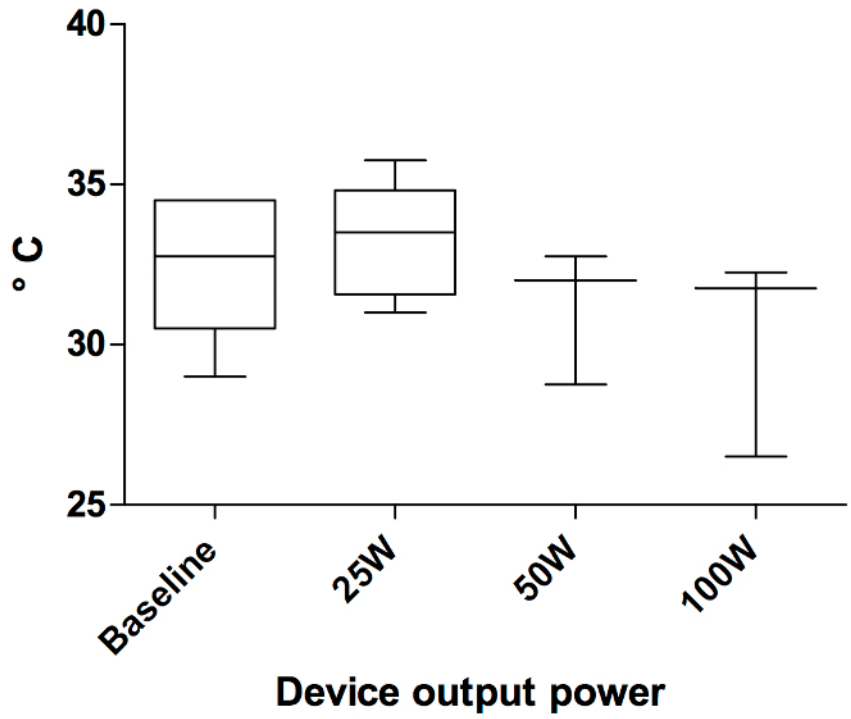


**Figure 6: Temperature at coagulation sites measured with an infrared thermometer.** The temperature at the coagulation sites measured with an infrared thermometer before and after argon plasma coagulation (n=3-6). Please click here to view a larger version of this figure.

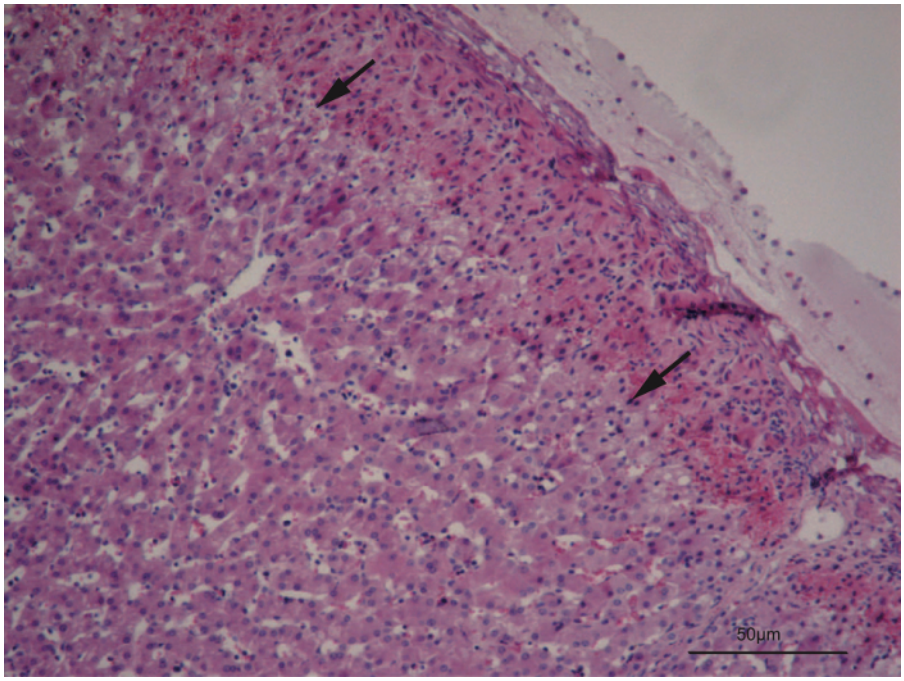


**Figure 7: Zone of superficial necrosis following helium plasma coagulation.** Hematoxylin/Eosin stained liver section at 40X magnification. The zone of necrosis shows a loss of hepatocyte cord architecture, cells with shrunken cytoplasm and hemorrhage zones. Arrows indicate the depth of coagulation at two different locations. Please click here to view a larger version of this figure.

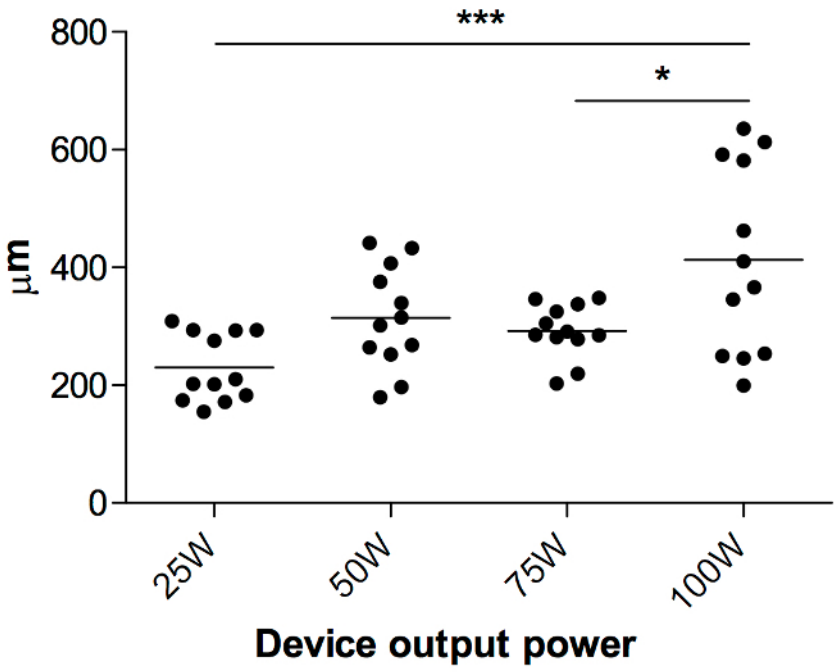


**Figure 8: Coagulation depth following helium plasma coagulation.** Coagulation depth at different power levels (25W, 50W, 75W and 100W, n=6). * = *P*<0.05, *** = *P*<0.001, 1-way ANOVA. Please click here to view a larger version of this figure.

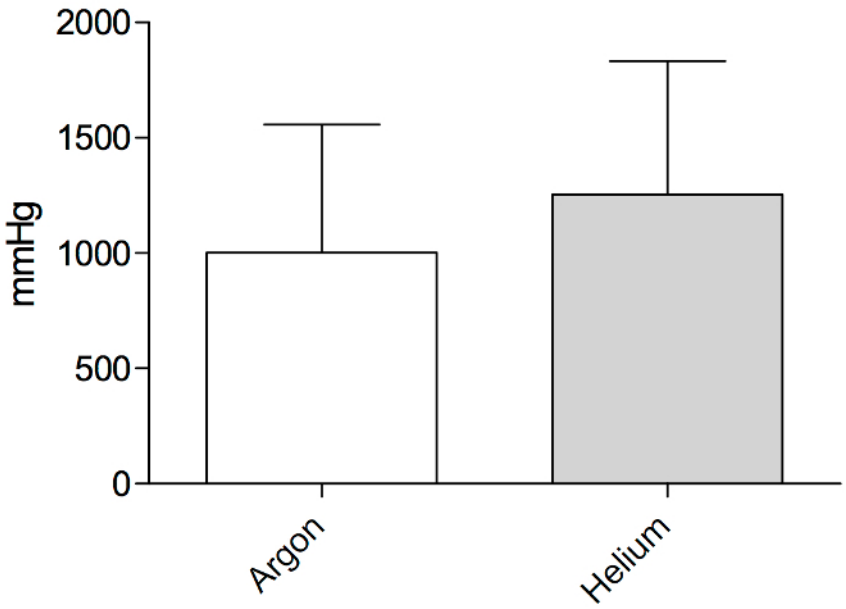


**Figure 9: Burst pressure.** Burst pressure measurements on the liver cut surface following either argon or helium plasma coagulation. Please click here to view a larger version of this figure.

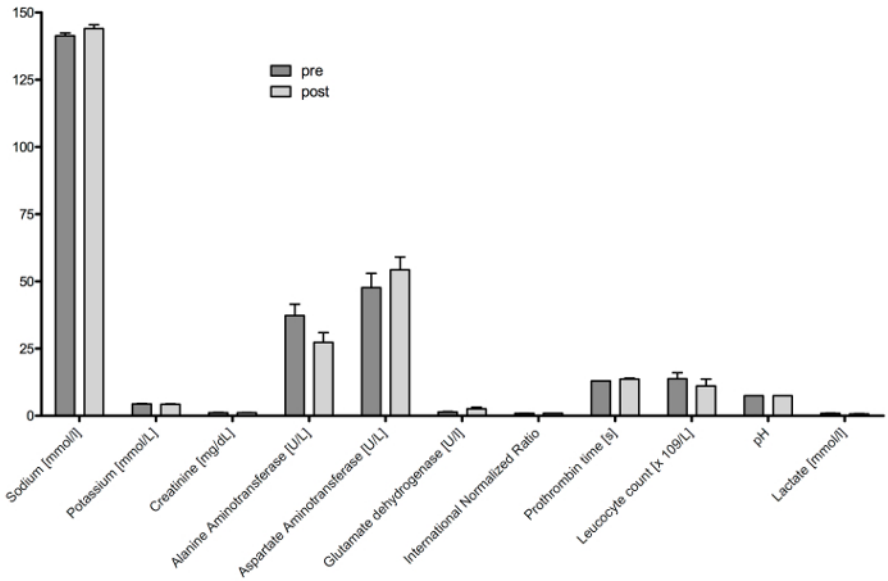


**Figure 10: Blood test results.** Selected parameters of clinical biochemistry and blood gas results are shown before and following argon plasma coagulation. No significant changes occur, demonstrating the effects of plasma coagulation limited to local changes at the coagulation site. Please click here to view a larger version of this figure.

## Discussion

Rodent models for liver surgery are established for a long time[16]. Nevertheless, large animal models offer certain advantages: no microsurgical equipment is needed as standard operative equipment for humans can be applied, surgical techniques are comparable to clinical use and standard clinical evaluation methods can be transferred to the experiments. For example, standard clinical blood tests can be carried out without the need for special laboratory test methods (**Figure 10**).

Swine are appropriate laboratory animals for cardiorespiratory research as their physiology closely resembles the human[17]. Because of the similarity in size, segmental structure and histology, pigs are also one of the standard laboratory animals for experimental hepatic surgery[18]. Plasma coagulation was evaluated in the porcine model because of the benefits (similarity to human physiology and evaluation of standard clinical equipment)[7]. In contrast to surgical techniques, anesthesiologic management cannot be easily extrapolated. Especially the airway management can be difficult[17]. The distance from the incisors to the glottis is very long and the anatomy is different to humans making

orotracheal intubation difficult for the inexperienced researcher. In addition, mask ventilation is nearly impossible in pigs, so salvage strategies (e.g. tracheostomy) should be present.

To achieve comparable results in plasma coagulation, the researcher should strictly take attention to standardize probe distance and duration of coagulation. While it is relatively easy to maintain the probe distance, a stopwatch can be used to count the 5 s of coagulation. The described technique of plasma coagulation on the liver surface was used in basic research on the underlying effects of plasma coagulation on the liver *in vivo*[7]. The above-described techniques of swine anesthesia, surgery, and plasma coagulation can also be used to examine major hepatic resection and to compare different techniques of cut surface sealing thereafter.

The laser Doppler flowmeter and the spectrophotometer for microcirculation measurements is a standard clinical tool[19] and proved to be highly useful for the assessment of circulation directly on the organ parenchyma. Values for blood flow and blood flow velocity are calculated with the advantage of non-invasivity. Microcirculation parameters are only indirect measures of the coagulation effect, so Doppler measurements should be correlated with an objective parameter for coagulation. In our experiments, we used histological coagulation depth for correlation.

A shortcoming of the temperature measurement is the inability to measure the temperature of the plasma beam during coagulation because the temperature of the plasma beam is above the upper threshold of both devices. The infrared thermometer is easy to apply, whereas the thermographic camera setup is more complex, but provides more precise data. The baseline temperature before coagulation is lower than expected (porcine body temperature ~38.5 ° C[17]), demonstrating the disruptive effects of laparotomy on body temperature. The measured temperature does not increase during and after coagulation, demonstrating the excellent perfusion of the liver. This thermal stealing effect of the liver is known from radio frequency ablation[20]. Burst pressure managements were conducted on the bile duct system rather than on hepatic vessels for a simple reason: it is impossible for plasma coagulators (as it is for fibrin sealants) to seal larger vessels. Both means of secondary hemostasis seal the cut surface of the resected organ, while larger vessels are ligated during resection. Our burst pressure experiments were slightly modified compared to the reported technique[14]. We measured burst pressure on explanted organs for organizational reasons. These rules out blood pressure related effects and are much easier to apply than to use perfused or *in-vivo* organs. Values of the pressure experiments might, therefore, differ from perfused/*in-vivo* measurements due to altered liver structure (usually higher pressures on explanted organs). The above-described burst pressure technique can also be performed *in-vivo*.

## Disclosures

The authors have nothing to disclose.

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