Aus dem

1) Interuniversitären Zentrum für medizinische Technologie

Stuttgart – Tübingen

in Kooperation mit der 2) Harvard Medical School und der

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Basic Principles and Future Aspects of Thermal Fusion and Electrocoagulation – Experimental studies in in-vitro and in-vivo rodent, porcine and human models

Inaugural-Dissertation
zur Erlangung des Doktorgrades
der Medizin

der Medizinischen Fakultät
der Eberhard Karls Universität
zu Tübingen

vorgelegt von

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aus

Homburg/Saar

2009

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1. INTRODUCTION

1.1. The introduction of laparoscopy

Laparoscopy was transformed from a diagnostic tool into a means for therapy by Kurt Semm, who performed the first laparoscopic appendectomy in 1983 [1]. Since then, increasing numbers of minimally-invasive laparoscopic procedures are being introduced into gynecological surgery [2, 3] and have become routine.

The clinical benefits of laparoscopy over laparotomy include shorter hospitalization, reduced pain, less blood loss, accelerates recovery, lowers expenses and decreased extent of adhesions [4].

1.2. The importance of bipolar vessel sealing in minimally invasive and open surgery

This success has depended upon continuous improvements in technology, with the introduction of electrosurgical vessel sealing probably being the most fundamental [2, 5, 6]. Vessel sealing during laparoscopic procedures with electrosurgical methods using bipolar current has been widely introduced over the past decade [2, 5, 7-9]. In the laparoscopic management of ovarian remnants for example, electrocoagulation with bipolar forceps for ablation of tissue was less traumatic and decreased the number of recurrences, conversions to laparotomy, and postoperative complications [10].

Bipolar, sealing-induced hemostasis can withstand high intraluminal pressures [5, 8, 11-15], and for many applications has been more efficient than any alternative ligation technique (e.g., suture, hemoclips, UCS) [11, 14] resulting in reduced blood loss [6, 16]. The seals are intrinsic to the vessel wall structure, are not adhesiogenic, and cannot be dislodged like some clips used for hemostasis [8, 12].

1.3. Limitations of bipolar vessel sealing

However, the introduction of energy-based vessel ligation has also been associated with complications, especially with more complex laparoscopic interventions [17, 18]. Firstly, the quality of vessel sealing, is often suboptimal [3, 7, 15] and manipulation to disengage the sealing instrument can weaken the seal [7]. Secondly, thermal injuries and ischemic injuries from direct heat exposure or thermal spread to adjacent tissues can induce hemorrhagic complications and tissue necrosis [2, 7, 19]. This can lead to, e.g., bowel injury, a feared complication [15, 19] that may go unrecognized during surgery and may not present until 3–14 days after surgery [2].

Often, the mechanism of thermal complications is not understood and essentially it is unclear whether thermal spread, careless manipulation with heated devices, or heated tissue causes the reported complications, e.g., ureterovaginal, vesicovaginal or duodenal fistulas, and rectal perforations [16, 20]. Thirdly, electrocoagulation is known to predispose to adhesion formation [21, 22] although it used extensively to achieve haemostasis. The relationship between thermal trauma and adhesion formation has not been studied in depth.

1.4. Objectives and rationales of the presented studies

The aims of the following studies were

1.4.1. To develop and test a new intelligent bipolar vessel sealing mode to improve the strength and reliability of the vessel seals in an in vitro porcine model.

Conventional pulsed bipolar coagulation (CPC) has been shown to lead to adequate vessel sealing [4, 5, 8, 10]. The methods used so far, however, have been based on a pulse frequency not regulated by impedance but dependent on a preset relationship between pulse and pause duration. As tissue

impedance increases due to thermal alteration, the current during the pulse decreases considerably and, after a certain point, even decreases to such an extent that the resulting heat energy, as dictated by Joule's Law, is too low to maintain the optimum tissue temperature needed for coagulation. During CPC, as tissue impedance continues to increase to higher levels, the fraction of the pulse during which current flow is not sufficient also lengthens.

We developed a new modulation of CPC where, unlike CPC, an electrical feedback mechanism based on the degree of denaturation and desiccation of tissue regulates the duration of pulses and pulse-pause sequences. As soon as current flow decreases to a defined level as a response to the increasing impedance, the pause and the next pulse are initiated automatically, thus avoiding longer fractions of pulses during which the current is too low to ensure the optimum tissue temperature. This dynamic time sequencing not only shortens the process as a whole, it also ensures a higher overall current. We termed this new modulation "intelligent", impedance-regulated, pulsed coagulation" (IPC). The aim of the present study was to establish whether the safety and reliability of vessel sealing with IPC is superior to CPC. Therefore we conducted the study "Intelligent, impedance-regulated, pulsed coagulation in a porcine renal artery model".

1.4.2. To analyse the biothermomechanics behind bipolar vessel sealing in order to better understand the process of thermal fusion in an in vitro poricine model.

Specific biothermomechanical parameters are key to the success of electrosurgical vessel sealing. In particular, the applied temperature and compressive pressure are thought to be pivotal factors [23, 24]. Additionally, tissue shrinking occurs during the sealing process. Very little detailed analyses of these parameters are available today. Moreover, it is not clear whether or not the applied high-frequency electrical current acts independently from the resulting increase in temperature or not. The current study evaluates the

influence of compressive pressure and temperature on electro-surgical vessel sealing in an in-vitro setup. We also investigated the relationship between achieving a good quality seal and mechanical vessel contraction. Finally, we reveal the effects of heat generated by purely thermal conduction as opposed to the high-frequency effects induced by electrical current. The underlying study was termed "Thermal conduction, compression and electrical current – an evaluation of major parameters of electrosurgical vessel sealing in a porcine in-vitro model".

1.4.3. To investigate the relationship between electrocoagulation and adhesion formation in an in vivo rodent model.

Adhesions occur after abdominal and pelvic surgery in over 70% of cases [25] and are generally believed to form secondary to peritoneal damage with a subsequent imbalance in peritoneal fibrinolysis [21, 26]. Patients who have developed post-operative adhesions are at risk of serious complications, including intestinal obstruction [27] and infertility [28]. If adhesiolysis is required, the affected patients are additionally exposed to the risks and complications of re-surgery and anaesthesia. Adhesions also place a burden on surgeons due to prolonged subsequent operations [29], which are potentially associated with greater risk of enterotomy [30]. Finally, there is a considerable financial burden on the health system with the cumulative costs over 10 years of adhesion-related readmissions in the United Kingdom estimated at £569 million [31].

Suturing was shown to induce adhesion formation and it has been hypothesised that this is secondary to ischemia [32]. Similarly, electrocoagulation is known to predispose to adhesion formation [21, 22]. Yet electrocoagulation is used extensively to achieve haemostasis. Therefore a thourough analysis of the relationship between electrocoagulation and adhesion formation is indicated. In the current study we investigate the hypothesis that the extent of trauma through electrocoagulation results in varying degrees of post-surgical adhesion and study the additive effect of suturing. The study was termed "The extent of

adhesion induction through electrocoagulation and suturing in an experimental rat study".

1.4.4. To establish a human in-vivo in-situ model to further analyse the basic mechanisms of thermal spread and thermal tissue damage as well as increasing safety of laparoscopic surgery.

In response to the unacceptable number of limitations and complications of electrocoagulation, numerous electrosurgical devices have entered clinical practice, including pulsed systems [14, 33, 34] or instruments with conductive paths inside their jaws [35]. Many instrumental factors such as jaw size and clamp surface [36] or compressive pressure during coagulation, maximum temperature and the dynamics of heat deposition into the tissue [37] have been found to be crucial to successful thermal fusion but they still await detailed investigation.

Thus, to date, choice of technique appears to be based much more on surgeon preference than on objective human data. In particular, thermal spread and thermal tissue damage have so far only been investigated in animal studies based on postoperative histological analysis of vessel seal samples or observation of the extent of birefringence loss [15, 17], and although there are isolated reports of in-situ measurements in animals [38], detailed investigations in a human in-vivo and in-situ model are still lacking. Overall, the widespread clinical use of electrocoagulation is not reflected in an equally detailed understanding of the underlying biothermomechanics [39] and the potential risks.

We therefore sought to establish a human in-vivo in-situ model for further analysis of the basic mechanisms of thermal spread and thermal tissue damage with a view to increasing the safety of laparoscopic surgery. For this purpose, we developed a model designed to generate comprehensive, easily reproducible, standardized data defining the thermal electrocoagulation-induced

effects on human tissue, based on four assessment categories: spatiotemporal changes in deep tissue temperature and tissue surface temperature; macroscopic scores; and microscopic scores. The study was termed "Quantifying electrocoagulation-induced thermal effects and damage to human tissue: An exploratory study using the fallopian tube as a novel in-vivo in-situ model".

2. MATERIAL AND METHODS

For all the above mentioned limitations of bipolar vessel sealing and electrosurgical devices in general, a series of studies was planned and performed in order to optimize seal quality with (1) an improved modulation of electric energy flow and (2) a better understanding of the biothermomechanics while at the same time (3) quantifying the adhesiogenic potential of bipolar vessel sealing and (4) developing a lasting model human in-vivo to investigate thermal spread and tissue defects in order to increase safety in electrosurgery.

Therefore, in a first step a new intelligent mode for optimized bipolar vessel sealing was developed and tested in an in-vitro animal model. With this mode, other variables such as temperature and pressure were analysed in the advanced form of the original in-vitro animal model. Thirdly, the adhesiogenic potential of the newly developed bipolar vessel sealing was assessed in an in-vivo animal model and finally, all findings were incorporated in the development of a human in vivo model for the investigation of thermal effects and tissue defects of electrosurgery.

2.1. Methodology Study 1: Intelligent, impedance-regulated, pulsed coagulation in a porcine renal artery model

2.1.1. Material and study design

The study was approved by the Research Programs Council of the University of Tübingen and the European Academy of the European Society of Gynecological Endoscopy.

Three different modulations of electrothermal energy were applied to test their efficacy in vascular coagulation of 132 renal arteries were harvested from female Swabian pigs. The vessels were randomly assigned to a specific coagulation mode. Two were CPC modes with a tissue-independent pulse frequency: pulse duration of 800 ms with a pause lasting 30 ms (CPC-I group; n=50) or 300 ms (CPC-II group, n=43). The third modulation (IPC group; n=20) was designed to be 'intelligent' and to regulate itself in a single coagulation procedure in response to the changing tissue impedance during thermal alteration (Figure 1). In line with clinical practice, some additional vessels underwent multiple coagulation (three times) with CPC-I and CPC-II (MCPC-I and MCPC-II). The investigation therefore included five groups of vessels.

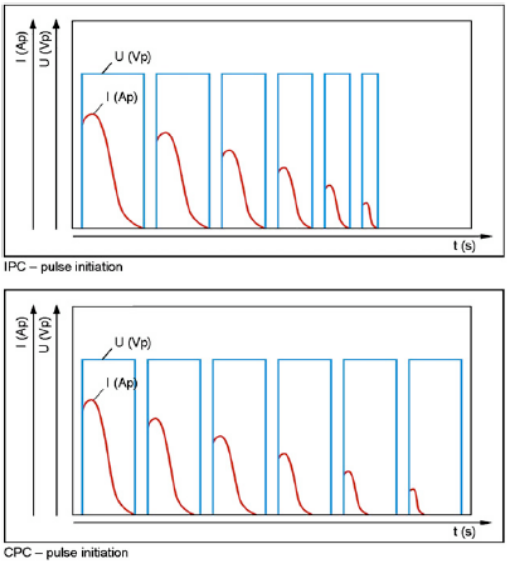

Figure 1. Intelligent Coagulation: Pulse initiation in IPC (upper panel) and CPC (lower panel). In IPC, the pulses are initiated when current flow decreases to a certain level. Pulse initiation is therefore impedance-dependent, while in CPC, pulse initiation is based on a predetermined relationship between pulse and pause.

2.1.2. Interventions

132 renal arteries were harvested from female Swabian pigs (weight range: 45–55 kg). The specimens were dissected and the diameters determined. The vessels were thoroughly flushed with normal saline to remove all blood. They were then stretched across a titanium adapter and secured with purse-string sutures to a pressure application device. Figure 2 shows the experimental setup.

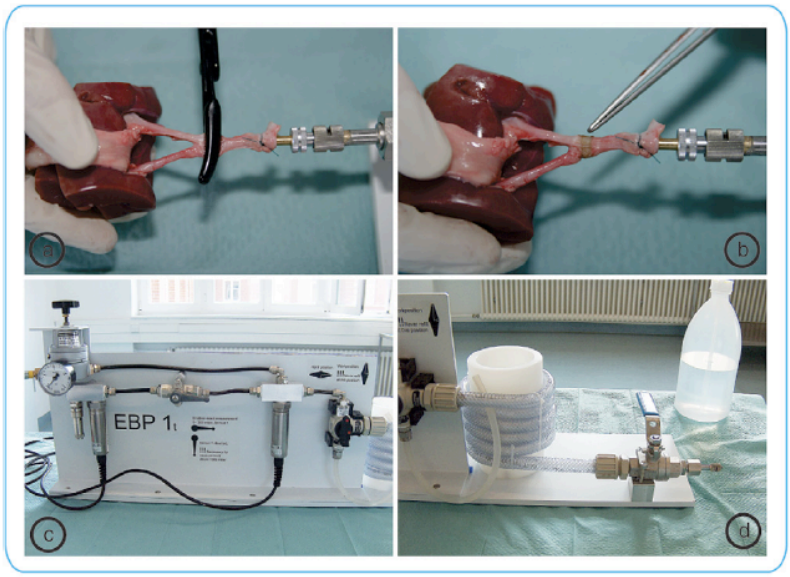

Figure 2. Intelligent Coagulation: Coagulation process (a and b) and pressure application device (c and d).

Vessel sealing was performed using bipolar open forceps (ERBE BiClamp) and a pulsed high-frequency generator (ERBE VIO 300 D). Coagulation with the two-jawed clamp was triggered via a pedal, and visual and audio signals indicated that the process was completed. The pedal was then released. The system generator and the surgical instrument used in the experiment are already in extensive use in laparoscopic surgery. The forceps pressure was standardized by a sprung handle. During the heating process, all relevant information on current, voltage and impedance were recorded digitally. The

completed seal was visually inspected for discoloring, flatness and translucency.

2.1.3. Measurements and parameters

After sealing, the burst pressure (BP) was determined as a measure of seal quality by a different operator, who did not know which mode had been used to seal the vessel. Saline was infused to gradually increase the perfusion pressure by 20 mmHg per second under constant pressure-monitoring until either the seal or the vessel wall burst. The pressure at which this occurred was defined as the burst pressure in mmHg. Vessels with seals that did not resist a pressure of 80 mmHg were considered instant seal failures and those that did not resist up to 200 mmHg were considered secondary seal failures. Vessels with seals that resisted a pressure of 200 mmHg were considered successful seals.

2.1.4. Statistical analysis

Data are given as mean ± standard error of the mean (SEM). After demonstrating normal distribution and homogeneity of variance across groups, differences between groups (burst pressure) were calculated by one-way analysis of variance (ANOVA) followed by an appropriate post-hoc test, including the correction of the alpha error to compensate for multiple comparisons. Fisher's exact test was used for the analysis of differences in rates and proportions (seal failures). Overall statistical significance was set at $p<0.05$. The Statistics Package for Social Sciences (SPSS), Version 11.5 for Windows (SPSS Inc., Chicago, USA) was used for statistical analysis.

2.2. Methodology Study 2: Thermal conduction, compression and electrical current – an evaluation of major parameters of electrosurgical vessel sealing in a porcine in-vitro model

2.2.1. Material and study design

The study protocol was approved by the Research Programs Council of the University of Tuebingen and the European Academy of the European Society of Gynecological Endoscopy.

A total of 106 porcine vessels (58 renal, femoral and carotid arteries with a mean calibre of 5.1 mm ranging from 2 to 8 mm and 48 veins with a mean calibre of 4.8 mm ranging from 2 to 8 mm) were harvested by the same investigator from 5 female Swabian-Hall pigs. The harvested vessels were randomised for the experiments. 59 vessels were used to investigate the correlation between compressive pressure during bipolar electro-coaptation and seal quality (Table 1). The remaining 47 vessels were used to study vessel sealing by purely thermal conduction (Table 1).

Table 1: Evaluation of major parameters of electrosurgical vessel sealing: Study Groups and Parameters.

Experiment	**Vessels**	**Group**	**Compres-sive Pressure (mN/mm²)**	**Number of Vessels**	**Calibre**	**Tem-perature**
Compressive pressure in bipolar electro-coaptation	Arteries	I-A1	120	11	4,7	Automatic
		I-A2	270	11	5,1	Automatic
		I-A3	380	10	5,2	Automatic
	Veins	I-V1	60	7	4,6	Automatic
		I-V2	120	6	4,2	Automatic
		I-V3	200	7	4,4	Automatic
		I-V4	270	7	4,5	Automatic
Coaptation through thermal conduction	Arteries	II-Arandom	300-800	10	5,1	140-220
		II-A1	500	4	5,5	125
		II-A2	500	4	5,3	155
		II-A3	500	4	5,5	185
		II-A4	600	4	5,1	155
	Veins	II-Vrandom	40-600	5	5,1	125-205
		II-V1	150	6	5,5	155
		II-V2	250	5	5,0	155
		II-V3	300	5	5,6	155

2.2.2. Interventions

An experimental setup (Figure 3) was designed that allowed standardised coaptation using either bipolar electrical current or purely thermal conduction without electrical current. The vertical compressive pressure during coagulation could be altered independently.

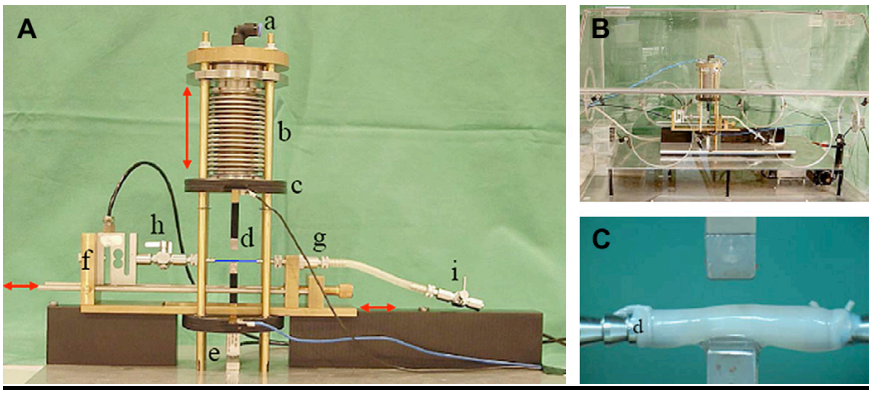

Figure 3. Evaluation of major parameters of electrosurgical vessel sealing: Experimental Setup. Panel A shows the setup with application of compressive pressure. Panel B depicts the experimental setup in the incubator. Panel C demonstrates a vessel in the coaptation device.

All experiments were preformed in an incubator at 36°C and 90% humidity to mimic physiologic conditions.

For the sealing process, 2 pairs of jaws with rounded edges, simple surface geometry and low thermal capacity were purposely-built to include temperature probes (Nickel-Chrome-Elements Type K, Reckmann Measurement Technology, Hagen, Germany) 0.1 mm beneath the surface. One pair of jaws, made from polished stainless steel, was connected to a Vio 300D bipolar generator (ERBE Electromedicin, Tuebingen, Germany).

The generator was used with the clinically established pulsed BiClamp Mode (automatically modulated sine-wave signal form with a fundamental frequency of 350 kHz as previously described by Wallwiener et al. [34] and comparable to Valleylab's LigaSure [14] and Gyrus' PlasmaKinetic [33] pulsed bipolar systems and auto-stop.

The other pair of jaws was made from a silver-silicon compound (Wielandin GmbH, Pforzheim, Germany) to include a platinum micro-heater (Heraeus Sensor-Nite GmbH, Kleinostheim, Germany) for vessel sealing by purely

thermal conduction. Here the coaptation process was stopped after all visual and acoustical signs of vaporization had ceased. Both pairs of jaws were built into a pressure device capable of impinging the vessel with a defined vertical compressive pressure. The jaws had a width of 6 mm, which defined the area of coagulation.

The compressive pressure was applied through a metal bellow (Hydra-Metallbalg, Witzenmann GmbH, Pforzheim, Germany), filled with precision-controlled compressed air. A resistance strain gauge (ME-Messsystem GmbH, Henningsdorf, Germany) served as a force sensor to detect changes as small as 0.01 Newton.

All vessels were carefully dissected from their connective tissues in-situ and thoroughly flushed with Custadiol©, a solution designed to protect transplants during transport, to remove any residual blood. The vessels then remained in a Custadiol© bath.

Immediately prior to sealing, the respective vessel was warmed in a 36°C saline bath and attached to the experimental apparatus by Luer-Lock-connectors (Volzer Medizintechnik, Tuttlingen, Germany). The vessel was then inflated with saline to a constant internal pressure of 100 mmHg for arteries and 30 mmHg for veins to unfold the collagen fibres and the endothelium. Subsequently, the vessels were assigned a number according to the order of harvesting and randomised in blocks to the experimental groups so that each group contained vessels from all of the pigs. Table 1 depicts the study groups.

2.2.3. Measurements and parameters

Burst pressure was determined as a measure of seal strength immediately after coaptation. Saline was infused gradually to increase the perfusion pressure by 20 mmHg per second until the seal burst. Vessels that did not resist a pressure greater 100 mmHg for arteries or 30 mmHg for veins were considered instant

seal failures. Those vessels that did not resist pressures of 250 mmHg for arteries or 80 mmHg for veins were considered secondary seal failures. Sustaining a pressure greater than 250 mmHg and 80 mmHg respectively was the definition of a successful seal. For calculation of the mean burst pressure, both successful seals and secondary failures were taken into account.

Additionally, compressive pressure, temperature and changes in longitudinal vessel tension were digitally recorded over the duration of the experiment with a measuring board (ME-2600i PCI, Meilhaus Electronic, Puchheim, Germany) and Labview 7.0 software (National Instruments, Austin, Texas, USA).

2.2.4. Statistical analysis

Differences between groups were analysed non-parametrically by the Wilcoxon Test or Kruskal-Wallis Test for continuous variables. Other variables were tested by the chi square test. Correlations between variables were investigated non-parametrically after Spearman. The significance level was set to 0.05. For post-hoc tests the significance level was adjusted according to Bonferroni. The statistical analysis was done with JMP version 5.1.2 by SAS Institute Inc.

2.3. Methodology Study 3: The extent of adhesion induction through electrocoagulation and suturing in an experimental rat study

2.3.1. Material and study design

Institutional Review Board approval was obtained. Adhesion induction was performed on a total of 35 female Wistar rats (Charles River Laboratories) in 5 different groups. All animals with a weight range of 220–280g were housed under standardized laboratory conditions that were in keeping with the European requirements.

2.3.2. Interventions

All operations to induce adhesions were performed by the same surgeon under aseptic conditions. Anaesthesia was induced by nebulised isoflurane, and intraperitoneal ketamine (100mg/kg) and xylazin (5mg/kg). The concentration of the injected ketamine was 100 mg/ml, and the concentration of the injected xylazin was 20 mg/ml. The peritoneal cavity was opened by a 4cm midline incision. Subsequently the animal was allocated to one of the 5 experimental groups (Table 2) according to a permutated block randomisation plan. Both lateral body walls of the animal were then traumatised accordingly. Per session, one animal from each group was operated.

Table 2: Electrocoagulation and adhesion formation: Study groups.

Group	Trauma	Number of traumatized areas	Number of animals
1	Minimal electrocoagulation	14	7
2	Extensive electrocoagulation	14	7
3	Minimal electrocoagulation + suturing of the underlying musculature	14	7
4	Traumatisation of the peritoneum only by mechanical denuding of the peritoneum	14	7
5	Traumatisation of the peritoneum only by mechanical denuding + suturing of the underlying musculature	14	7

Adhesion induction (Figure 4): All traumatisation was inflicted by the same surgeon. Standardisation of the traumatised area was achieved using rectangular plastic stencils with cut-out centres of the sizes of the intended traumata. The applied pressure was standardised using electronic scales, which were placed underneath the tissue being traumatised.

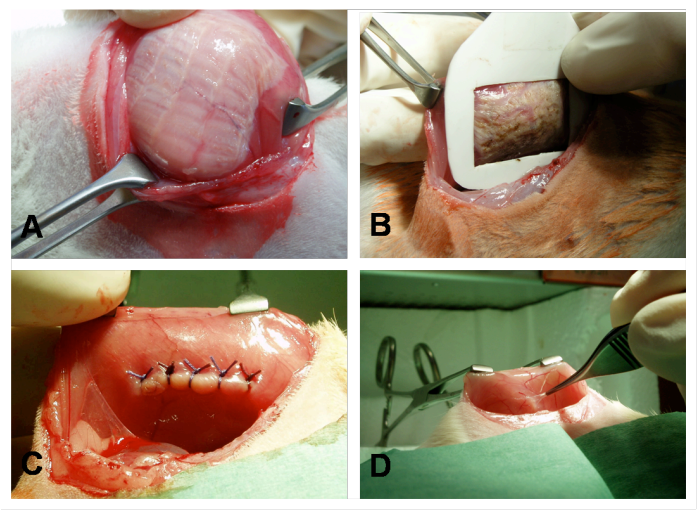

Figure 4. Electrocoagulation and adhesion formation: Adhesion induction. Minimal coagulation (Panel A), extensive coagulation (Panel B), minimal coagulation plus suturing (Panel C), mechanical peritoneal denuding (Panel D).

In group 1, minimal electrocoagulation, standardised lesions were inflicted over an area of 2.5 x 2 cm by sweeping bipolar forceps over the abdominal peritoneum. At this, the forceps were fixed open so that both branches and open distances measured exactly 0.5 cm and each sweeping was done exactly below the prior one. The time for each sweeping was 1 second and the pressure that was applied on the tissue via the forceps during each sweeping amounted to 15g. The generator was set to 60 Watts. For all electrocoagulation, bipolar coagulation forceps (Coagulationforceps “normal length”, ERBE Elektromedizin, Germany) and a Vio 300D bipolar generator (ERBE Elektromedizin, Germany) were used which are among the standard instrumentation in our hospital.

In group 2, extensive electrocoagulation, traumatisation was achieved similarly to group 1 but each sweeping lasted 3 seconds and the pressure on the tissue amounted to 45g. In group 3, narrow stripes of 2 x 0.5 cm area were created as for group 1 but with additional suturing through the underlying musculature approximately 1 mm deep, with five interrupted sutures (3/0 polyglactin, Ethicon) placed equidistantly over the peritoneal defect [40, 41]. The tension of

the sutures was chosen to simulate approximation of the peritoneum during wound closure. In group 4, the peritoneum was carefully incised and stripped off the musculature over an area of 2.5 x 2 cm. In group 5 narrow stripes of 2 x 0.5 cm area were created as for group 4 but with with additional suturing as in group 3.

These models were chosen to replicate the different aspects of peritoneal trauma during surgery (electrocautery, suturing, sharp incision and mechanical damage). The relatively large area of 2.5 x 2 cm chosen to examine adhesion formation after minimal electrocoagulation or extensive electrocoagulation was decided upon because previous pilot experiments of denuding or minimal electrocoagulation resulted in little adhesion formation.

In order to combine the modalities electrocoagulation and suturing and thereby mimicking the situation in the human operating theatre, smaller areas were coagulated, thus, enabling the surgeon to overstitch the lesion. The smaller traumatized area was no concern for the authors, since previous pilot experiments led the authors to believe that suturing would greatly increase adhesion formation [40, 41].

Complete hemostasis was achieved using pressure from a sterile swab. Subsequently the midline incision was closed in two layers with continuous 3/0 polyglactin. The duration of each surgery was approximately 20 minutes from incision to closure of the skin. Post-operatively the animals received 0.05ml buprenorphine (0.05-0.1 mg/kg) subcutaneously as soon as the animals' whiskers started moving after the operation and then 4 times per day for 3 days. Afterwards the animals were observed daily for signs of complications. After 14 days the animals were sacrificed using CO2.

2.3.3. Measurements and parameters

Adhesion scoring: The adhesion scoring was performed immediately after euthanasia by a pathologist to whom the allocation of each animal was blinded. Adhesion incidence in percent was defined as the number of trauma sites at which adhesions developed post-surgically. Adhesion quantity was defined as the adhesion-covered area divided by the area of the traumatized area. Adhesion quality was considered “filmy” if the scale of a ruler was visible through the tissue, otherwise it was considered “dense” [40].

Histopathology: All traumatised areas were excised en-bloc together with any adhesive tissue and fixed in 4% PBS-buffered formalin. After routine tissue processing, histological evaluation was done by hematoxilin/eosin staining, Elastica van Gieson and Goldner staining for fibrous tissues as well as Pears staining for fibrin. Histological analyses were performed by Dr. Christoph Brochausen at the Department of Pathology at the University of Mainz, Germany.

2.3.4. Statistical analysis

Adhesion incidence was analysed using Fisher’s Exact test. Statistical significance in adhesion quantity and quality was tested using Kruskal-Wallis analysis with Bonferroni correction to protect the overall error rate against multiple significance tests. The significance level was set to Alpha = 0.05. Error bars represent the 95% confidence interval. The statistical analysis was done with a statistics package (JMP, Version 5.2.1; SAS Institute Inc., Cary, NC) and R (R Development Core Team, Vienna, Austria).

2.4. Methodology Study 4: Quantifying electrocoagulation-induced thermal effects and damage to human tissue: An exploratory study using the fallopian tube as a novel in-vivo in-situ model

2.4.1. Material and study design

This was a prospective, open, uncontrolled, non-randomized, single-center exploratory study. The study protocol was approved in advance by the Ethics Committee of the Medical Faculty of the University of Tübingen, Germany as project number 425/2006M. Included were 18 patients older than 18 years who had already consented to, and were scheduled for, abdominal hysterectomy by laparotomy for benign disease. Patients were recruited consecutively, confirmed their ability and willingness to comply with study procedures and gave their written informed consent. All surgery was performed at the Department of Obstetrics and Gynaecology of the University of Tübingen, Tübingen, Germany.

Work-up for surgery and general anesthesia during surgery were performed according to standard in-house procedures and documented in the patient records. Surgical exposure of the uterus and the fallopian tubes was performed in a routine fashion. The experimental interventions were carried out prior to resection of the fallopian tubes. The operation was then completed in the usual manner.

2.4.2. Interventions

Per patient, one fallopian tube was grasped and coagulated for 10 seconds with a laparoscopic bipolar clamp (Robi® Laparoscopic Forceps 38221 ON, KARL STORZ GmbH & Co. KG, Tuttlingen, Germany). Energy was supplied by a VIO 300D electrosurgical unit (ESU; ERBE Elektromedizin GmbH, Tübingen, Germany), set to 40 Watts, Effect 5, and “Bipolar Soft” mode. Equipment, instruments and settings were routinely used for laparoscopic surgery in our hospital. The setup is depicted in Figure 5.

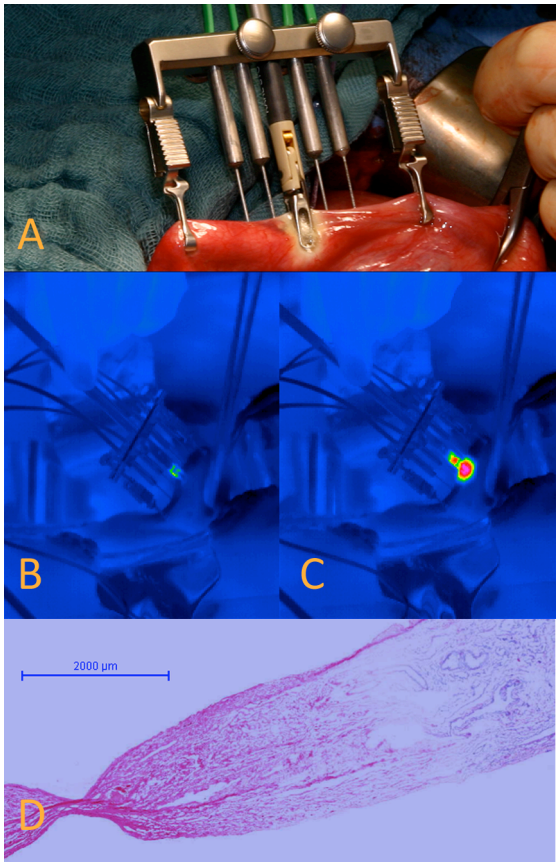

Figure 5. Quantifying thermal effects in a human in-vivo in-situ model: Experimental setup and tissue specimen. (A) electrocoagulation device comprising a bipolar clamp (center) and two lateral pairs of temperature probes; images taken with the thermal camera at the beginning (B) and during (C) electrocoagulation; and (D) LDH activity in a coagulated fallopian tube.

2.4.3. Measurements and parameters

Temperature measurements: *Deep tissue temperature* was measured using four thermal probes (NiCr-Ni sheathed thermocouple assemblies (MTE) 1_R 9-13®, Reckmann Mess & Regeltechnik, Hagen, Germany) that were custom-built into standard 0.7 mm diameter needles. The probes were fixed to a linear guide rail and inserted directly into the center of the fallopian tube. Two probes were located at 4.5 mm and the other two at 11.5 mm to the left and right of the edges of the bipolar forceps (equivalent to 7 mm and 14 mm from the middle of

the forceps). Deep tissue temperature was recorded at a rate of 1 Hz using Jumo Logoscreen CF equipment (Version 172.02.xx; JUMO, GmbH & Co. KG, Fulda, Germany) and the appropriate software for temperature monitoring and analysis (Version 2.06J). Prior to the study, the Safety Committee for medical equipment of the University of Tübingen confirmed that the use of the thermal probes raised no medical concerns according to the relevant standards, DIN VDE 0751-1 and DIN EN ISO 14791. Temperatures were recorded from 10 seconds before until 30 seconds after coagulation was initiated.

Tissue surface temperature was studied using a precalibrated high-specification thermal imaging camera (VarioCAM® fitted with an LW IR 1.0/25 mm lens, JENOPTIK Laser, Optik, Systeme GmbH, Jena, Germany). The camera was set up outside the sterile surgical area on a tripod-mounted swing arm and positioned 0.5 m above the surgical field at an angle of 90° to the fallopian tube fixed in the rail. The equipment was operated by experienced users. Thermal images were taken on verbal command 1 second before and 1, 10, 20 and 30 seconds after the start of the coagulation. The camera operated in the mid-infrared (8–13 µm) waveband and captured fully digitized 16-bit thermographic frames. Using the stored picture data, temperatures in each frame were later measured and analyzed with the IRBIS® Plus software (Version 2.2, InfraTec GmbH, Dresden, Germany).

Macroscopic analysis: As soon as the tube was resected, the coagulation site was described macroscopically in a quantitative, standardized fashion in consensus by two surgeons according to previously reported clinical criteria [36] (Table 3).

Table 3: Macroscopic and microscopic scores.

Evaluation	Criteria	Score
Macroscopic*	Tissue clarity	0 = not translucent; 1 = slightly translucent; 2 = moderately translucent; 3= fully translucent
	Tissue desiccation	0 = wet; 1 = slightly dried; 2 = moderately dried; 3 = fully dried
	Tissue charring	0 = no charring; 1 = few black spots; 2 = confluent black spots; 3 = completely black tissue area
	Instrument sticking	0 = no sticking; 1 = sticking but easy to remove instrument; 2 = sticking and difficult to remove instrument
Microscopic	Epithelial alteration	0 = none; 1 = some; 2 = moderately prevalent; 3 = frequent
	Tissue fragmentation	0 = none; 1 = some; 2 = moderately prevalent; 3 = frequent
	Tubal architecture	0 = no loss of architecture; 1 = loss of separation between endosalpinx and myosalpinx; 2 = inner circular and outer longitudinal myosalpinx undiscernible; 3 = complete loss of structure in endosalpinx, myosalpinx and serosa
	Basophilia	0 = no basophilia; 1 = slight basophilia; 2 = moderate basophilia; 3 = marked basophilia

* based on [36]

Histological analysis: The fallopian tube was cut longitudinally and stained using (a) hematoxylin and eosin and (b) the staining method for lactate dehydrogenase (LDH) activity described by Sherwood and Flotte [42]. In LDH-stained slides, thermal artefacts such as the distance from the edge of the cauterized zone to the beginning of undamaged stroma or epithelium were measured both inside the tube, at the epithelial level, and on the tubal surface.

To avoid describing thermal artefacts such as homogenous darkly stained tissue, tissue fragmentation and epithelial destruction [43, 44] and streaming artefacts with smudged chromatin and elongated, hyperchromatic nuclei, and

vacuolated signet cells of stromal derivation [45], we decided on the following four criteria for standardized and easily reproducible description of the microscopic changes: (1) epithelial alteration, (2) presence of tissue fragmentation as a differentiation between intact and detached internal mucosa (endosalpinx), (3) loss of histological tubal architecture due to electrocoagulation and (4) basophilia due to increased binding of hemalum following thermal damage. These criteria were assigned scores from 0 to 3 (Table 3).

All microscopic slides were evaluated and scored in consensus by two different blinded observers. Histological analyses were performed by Dr. Peter Fritz in the Department of Pathology at the Robert-Bosch-Krankenhaus Stuttgart, Germany and by Prof. Thomas Flotte in the Department of Pathology at the Mayo Clinic Minnesota, USA.

2.4.4. Statistical analysis

Data were analyzed using the R software, Version 2.7.2 (R Foundation for Statistical Computing, Vienna, Austria [46]. The strength of relationship between two ordinal or continuous variables was estimated using Spearman's rank correlation coefficient r_s. The two methods for measuring temperature, i.e. thermal camera and thermal probes, were compared using the Bland-Altman method [47]. The temperature distribution in space and time can be described by the heat equation. Therefore we expected that the one-dimensional temperature profile would roughly follow $b_1 \exp(-x^2/b_2)$, where x is the variable describing space, in our case the distance from the coagulation site. The parameters b_1 und b_2 were estimated by nonlinear regression. Estimation was done separately for each time point to allow for continued heating during the first 10 seconds.

3. RESULTS

3.1. Results Study 1: Intelligent, impedance-regulated, pulsed coagulation in a porcine renal artery model

3.1.1. Application of single CPC versus single IPC

In the first part of the study, single CPC-I (n=50) and single CPC-II (n=43) were compared with single IPC (n=20). The mean vessel diameter did not differ between the three groups ($p>0.05$) (Table 4). The mean burst pressure achieved after IPC (585.5 ± 56.8 mmHg) was significantly higher ($p<0.001$) than that measured after CPC-I (372.6 ± 40.0 mmHg) and CPC-II (334.2 ± 44.2). The pressures achieved in CPC-I-sealed arteries did not differ significantly from those observed in CPC-II-sealed arteries ($p>0.05$).

In the IPC group, instant seal failures were seen in 5.0% of the vessels studied, and successful sealing was observed in 95.0%. In contrast, CPC-I produced 28.0% instant and 6.0% secondary seal failures, and only 66.0% of the single CPC-I seals were considered successful. Similar results were found for CPC-II which produced 32.6% instant seal failures and 7.0% secondary seal failures, and only 60.5% of the single CPC-II seals were considered successful. Thus, the overall seal failure after IPC was significantly ($p<0.05$) lower than that observed after CPC-I and CPC-II (Table 4).

Table 4. Intelligent Coagulation: Coagulation of porcine renal arteries with conventional pulsed coagulation compared to intelligent, impedance-regulated, pulsed coagulation.

Data are mean ± SEM. CPC-I: conventional pulsed coagulation with 800 ms pulse/30 ms pause; CPC-II: conventional pulsed coagulation with 800 ms pulse/300 ms pause; IPC: intelligent, impedance-regulated, pulsed coagulation (self-adaptation to tissue impedance during thermal alteration). #p=0.05; *p<0.05 versus ICP

Coagulation mode	CPC-I	CPC-II	ICP
Total number of vessels [n]	50	43	20
Mean vessel diameter [mm]	4.52 ± 0.16	4.50 ± 0.17	4.20 ± 0.29
Instant seal failures [n (%)]	14 (28.0)#	14 (32.6)*	1 (5.0)
Secondary seal failures [n (%)]	3 (6.0)	3 (7.0)	0 (0.0)
Overall seal failures [n (%)]	17 (34.0)*	17 (39.6)*	1 (5.0)
Successful seals [n (%)]	33 (66.0)*	26 (60.5)*	19 (95.0)
Mean burst pressure [mmHg]	372.6 ± 40.0*	334.2 ± 44.2*	585.5 ± 56.8

3.1.2. Application of multiple CPC versus single IPC

In the second part of the study, multiple CPC was compared with the results of single IPC (n=20) (Table 5). Multiple CPC consisted of applying the coagulation mode three times in repetition. A total of 19 vessels were subjected to multiple coagulation, nine of them with CPC-I and ten of them with CPC-II. The mean vessel diameter did not differ between the three groups (p>0.05). The mean burst pressures achieved by these modes of coagulation did not differ significantly from that observed after single coagulation with IPC (p>0.05). In addition, both multiple CPC-I and multiple CPC-II did not produce any instant or secondary seal failures. Accordingly, the rate of seal failures of multiple CPC was not different from that observed after single ICP.

Table 5. Intelligent Coagulation: Coagulation of porcine renal arteries with multiple conventional pulsed coagulation compared to intelligent, impedance-regulated, pulsed coagulation.

Data are mean ± SEM. m-CPC-I: multiple conventional pulsed coagulation with 800 ms pulse/30 ms pause; m-CPC-II: multiple conventional pulsed coagulation with 800 ms pulse/300 ms pause; IPC: intelligent, impedance-regulated, pulsed coagulation (self-adaptation to tissue impedance during thermal alteration). Data are not significantly different between the three groups.

Coagulation mode	**m-CPC-I**	**m-CPC-II**	**ICP**
Total number of vessels [n]	9	10	20
Mean vessel diameter [mm]	4.22 ± 0.39	4.25 ± 0.37	4.20 ± 0.29
Instant seal failures [n (%)]	0 (0.0)	0 (0.0)	1 (5.0)
Secondary seal failures [n (%)]	0 (0.0)	0 (0.0)	0 (0.0)
Successful seals [n (%)]	9 (100.0)	10 (100.0)	19 (95.0)
Mean burst pressure [mmHg]	597.3 ± 60.1	656.2 ± 56.5	585.5 ± 56.8

3.1.3. Relationship between burst strength and vessel diameter

A clear inverse relationship between burst pressure and vessel diameter was seen. The mean burst pressure of sealed blood vessels with diameters below 4 mm was 528.0 ± 58.6 mmHg. Renal arteries with diameters larger than 4 mm showed a significantly ($p<0.05$) lower burst pressure when compared with that measured in the smaller blood vessels (Figure 6).

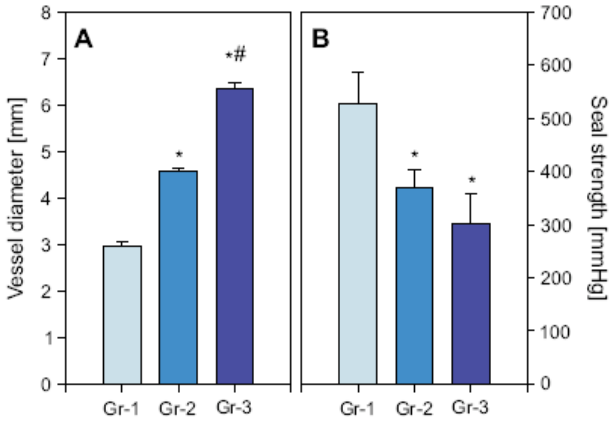

Figure 6. Intelligent Coagulation: Seal strength (B) given in relation to vessel diameter (A) after conventional or intelligent pulsed coagulation of porcine renal arteries.

Data are grouped according to vessel diameter, i.e. vessels with diameters <4 mm (Group 1, light blue bars), ³4 mm and <6 mm (Group 2, semi blue bars), and ³6 mm (Group 3, dark blue bars). Note the inverse relationship, indicating decreasing seal strength with increasing vessel diameters. Data are mean ± SEM; *p<0.05 versus Group 1, #p<0.05 versus Group 2.

3.2. Results Study 2: Thermal conduction, compression and electrical current – an evaluation of major parameters of electrosurgical vessel sealing in a porcine in-vitro model

There were no statistically significant differences in vessel calibre between the test groups. Neither did the anatomic origin of the vessels have any significant influence on the experimental outcomes or correlation with another study parameter nor did an individual animal.

3.2.1. Seal failures after bipolar electro-coaptation with different compression pressures (CP)

In arteries, the incidence of all seal failures (both initial and secondary) after bipolar coaptation was 72.7% for a CP of 120 mN/mm² (I-A1), falling to 0% for a

CP of 270 mN/mm² (Group I-A2) and then raising again to 20.0% for 380 mN/mm² (I-A3). These differences were statistically significant (p=0.002). In veins the differences in seal failures for different compressive pressures were not statistically significant for the number of samples studied (p>0.05) (Table 6).

Table 6: Evaluation of major parameters of electrosurgical vessel sealing: Compressive Pressure in Bipolar Coaptation.

	Group	Compressive Pressure mN/mm²	Number of Vessels	Successful Seals (SS)	Initial Failures (IF)	Secondary Failures (SF)	Maximum Temperature (°C)	Delta in Vessel Tension Start-End (N)
Arteries	I-A1	120	11	3	4	4	100 ± 7	0,7 ± 0,4
	I-A2	270	11	11	0	0	119 ± 5	1,8 ± 1,1
	I-A3	380	10	8	2	0	126 ± 4	1,4 ± 0,7
Veins	I-V1	60	7	4	1	2	110 ± 6	0,7 ± 0,6
	I-V2	120	6	4	0	2	115 ± 7	0,9 ± 1,1
	I-V3	200	7	3	3	1	127 ± 10	2,6 ± 1,1
	I-V4	270	7	4	2	1	127 ± 11	2,4 ± 0,8

3.2.2. Burst pressures (BP) after bipolar electro-coaptation with different compressive pressures (CP)

In arteries, the mean BP was 243±83 mmHg for CP of 120 mN/mm² (I-A1), increasing to 510±138 with CP of 270 mN/mm² (I-A2; p=0.0075) and then falling back to 375±103 mmHg with CP of 380 mN/mm² (I-A3; p=0,05). These differences were statistically significant (p=0.022) (Figure 7).

In the venous samples the differences between the individual groups were not statistically significant for the number of samples studied. For both arteries and veins, the combined BP for the intermediate groups were compared with the combined BP for the extreme groups.

For veins, the BP of the extreme groups (I-V1 (60mN/mm²) and I-V4 (270mN/mm²)) was 147±51 mmHg which contrasted significantly with 290±95 mmHg for the intermediate groups (I-V2 (120mN/mm²) and I-V3 (200mN/mm²)) (p=0.017). For arteries, the BP for the intermediate group I-A2 (270mN/mm²), 510±138 mmHg was significantly higher than 309±73 mmHg for the extreme groups I-A1 (120mN/mm²) and I-A3 (380mN/mm²) (p=0.022).

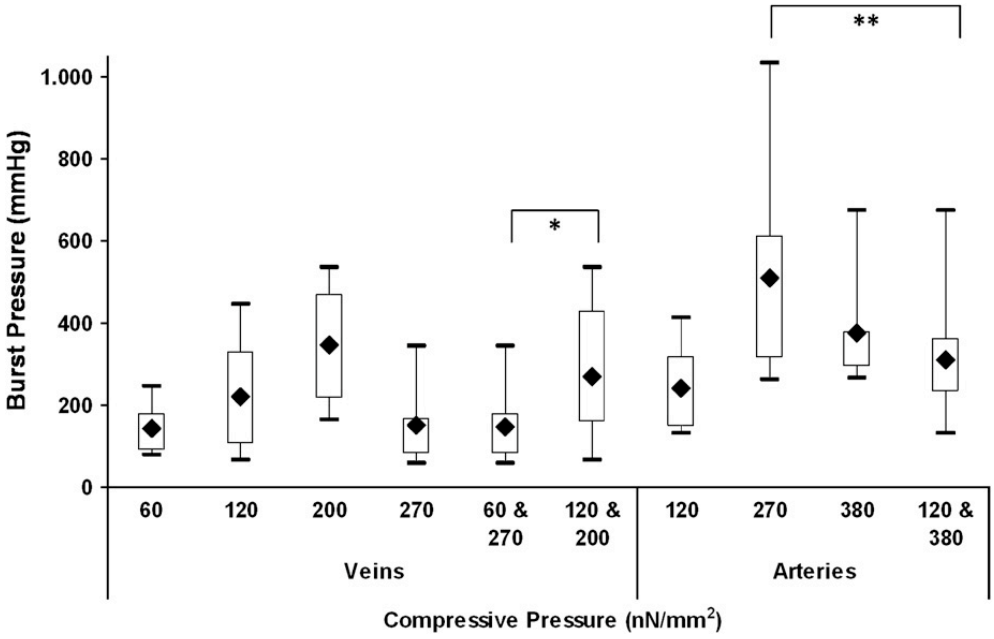

Figure 7. Evaluation of major parameters of electrosurgical vessel sealing: Burst pressures (BP) in bipolar vessel sealing with varying compressive pressures.
Mean BP in mmHg is shown for each group. Means (diamonds), interquartile ranges (boxes) and maximum and minimum values (error bars) are indicated. * p=0.017, ** p=0.022.

3.2.3. Maximum Temperature

Analysis of the maximum temperature (mT) values for seal failures and successes from all groups with bipolar vessel sealing revealed significant

differences. In arteries, the mean mT of all successful bipolar seals, 119±4,0°C, was significantly higher than the mean mT of all seal failures, 105± 10,0°C (p=0.011). In contrast, the mean mT for successful bipolar seals in veins, 116±7°C, was significantly lower than the mean mT for seal failures, 127±7°C (p=0.038) (Figure 8). Also, in both arteries and veins, mT correlated positively with CP (correlation coefficient after Spearman: rho=0.83 and p<0.001 in arteries and rho=0.54 and p=0.004 in veins) (Table 6).

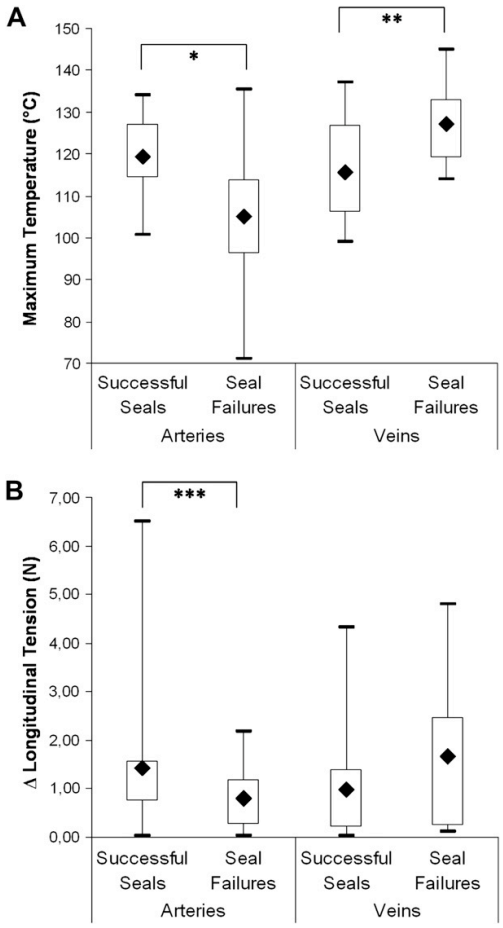

Figure 8. Evaluation of major parameters of electrosurgical vessel sealing: Correlation between sealing success and maximum temperature (MT) respectively changes in longitudinal vessel tension (ΔLT).
Panel A: Mean MT is shown in °C for all successful seals and all seal failures broken down by arteries and veins. Panel B: Mean ΔLT is shown in N analogue

to Panel A. Means (diamonds), interquartile ranges (boxes) and maximum and minimum values (error bars) are indicated. * p=0.011, ** p=0.038, *** p=0.019.

3.2.4. Changes in longitudinal tension

The changes in longitudinal tension exerted by the vessels for arteries and veins were calculated for successful seals as well as for seal failures after bipolar electro-coaptation and coaptation by purely thermal conduction. The mean difference in longitudinal tension before and after coaptation (∆lT) for successful arterial seals was 1.4±0.5 N. In contrast, the tension change in seal failures was only 0.8±0.2 N. These differences were significant (p=0.019) (Figure 8). For veins, the mean difference in ∆lT between successful seals and failures was not statistically significant (p>0.05) (Table 6).

3.2.5. Vessel sealing with coaptation by purely thermal conduction

Out of 26 arteries sealed purely by thermal conduction without electrosurgical effects, there were only 5 successful seals (19.2%). Sealing of veins by purely thermal conduction resulted in 11 successful seals out of 21 (52.4%). Thus vessel sealing by thermal conduction was less successful than bipolar electro-coaptation for equivalent temperatures. There was no statistically significant difference in the average temperature of the successful seals (167±28°C in arteries and 162±11°C in veins) compared to the average temperature of seal failures (164±12°C in arteries and 154±8°C in veins) (p>0.05).

3.3. Results Study 3: The extent of adhesion induction through electrocoagulation and suturing in an experimental rat study

3.3.1. Adhesion incidence

Figure 9a: Adhesions developed in 14% (n=2) of areas traumatised by minimal electrocoagulation. There were adhesions at all traumatized sites after

extensive electrocoagulation (n=14) and in the two groups involving suturing (n=14, n=14). There were no adhesions in the mechanical denuding group. Fisher's Exact Test revealed that these differences were highly significant (p<0.001).

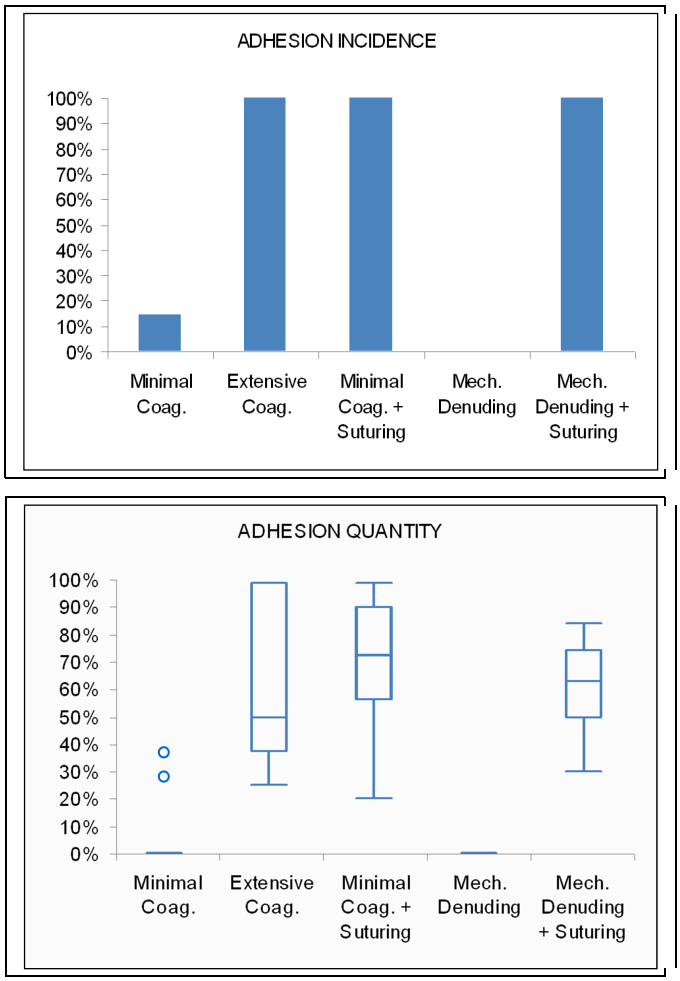

Figure 9. Electrocoagulation and adhesion formation: Adhesion Incidence and Quantitiy.

Shown is the incidence of post-surgical adhesion formation (Panel A, bars represent the adhesion formation in percent of traumata for each group) and the adhesion quantity (Panel B, the boxplots indicate minimum observation, first quartile, median, third quartile and maximum observation. According to standard convention observations 1.5 times the interquartile range lower than

the first quartile or 1.5 times the interquartile range higher than the third quartile are considered outliers and indicated by circles).

3.3.2. Adhesion Quantity

Figure 9b: Minimal electrocoagulation caused a mean adhesion quantity of 0% (range 0%-37%). Extensive electrocoagulation led to 50% adhesion quantity (range 25% - 100%). This difference was highly significant ($p<0.001$). Minimal electrocoagulation with additional suturing of the underlying tissue resulted in 73% adhesion quantity (range 20% - 100%). Here the difference against minimal electrocoagulation alone was also highly significant ($p<0.001$).

Peritoneal denuding caused no adhesions (range 0%-0%). If the underlying musculature was also sutured there was 64% adhesion quantity (range 30% - 85%). This difference was also highly significant ($p<0.001$). There was no statistically significant difference between denuding and minimal electrocoagulation.

3.3.3. Adhesion quality

The omental and pelvic fat were the only tissues attached to the traumatised areas with the only exception of two cases when intestine was included after extensive coagulation. Electrocoagulation trauma only, both minimal and extensive, resulted in only dense adhesions. After minimal electrocoagulation plus suturing dense adhesions covered 26% (range 0% - 83%) of the traumatised areas whereas filmy adhesions covered 39% (range 0% – 94%).

After peritoneal denuding plus suturing dense adhesions covered 35% (range 10% -75%) of the traumatised areas whereas filmy adhesions covered 15% (range 0% - 55%). Kruskal-Wallis one-way analysis of variance demonstrated significant differences between the groups ($p<0.001$).

3.3.4. Histopathology

Extensive electrocoagulation revealed damage not only to the serosal membrane but it also with affected the subserosa and the underlying musculature (in case of extensive coagulation). Moreover, edema in the subserosal connective tissue, hyperaemia of the small vessels and leukocytic infiltrate of the subserosa including the underlying musculature with destruction of muscle cells and beginning fibrous organisation could be demonstrated.

These effects were rarely seen with minimal electrocoagulation but in all cases of extensive coagulation. Animals with mechanical peritoneal denuding showed no changes in the underlying musculature. Animals with suturing had granuloma formation and foreign-body reaction in the musculature.

3.4. Results Study 4: Quantifying electrocoagulation-induced thermal effects and damage to human tissue: An exploratory study using the fallopian tube as a novel in-vivo in-situ model

Complete data sets were recorded for 15 fallopian tubes from the 18 patients who underwent surgery. For 2 patients, fallopian tubes were not available because they were used to diagnose the underlying disease. In the third case, the thermal camera was not available.

3.4.1. Maximum temperature, lateral thermal damage, and caliber of the fallopian tube

Maximum temperature rise (MTR) and mean lateral thermal damage (mLTD) as revealed by histology were strongly correlated (r_s = 0.93). Both MTR and mLTD demonstrated a moderate negative correlation with the tubal caliber (TC) (r_s = −0.64 for MTR and TC, and r_s = −0.56 for mLTD and TC). All other microscopic criteria were compared with the mLTD as the gold standard for the detection of thermal damage in this setting.

3.4.2. Deep tissue temperature

The data recorded at the center of the tube at 4.5 and 10.5 mm from the edge of the clamp are shown in Figure 10. The mean initial temperature (± SD; range) was 31.3°C (± 2.1°C; 28.1–34.3°C). At 4.5 mm distance, mean temperatures were > 60°C and > 50°C for > 4 seconds and 10 seconds, respectively. The mean MTR at 10.5 mm from the coagulation site was 5.0°C. For both distances, MTR was reached after 4 seconds.

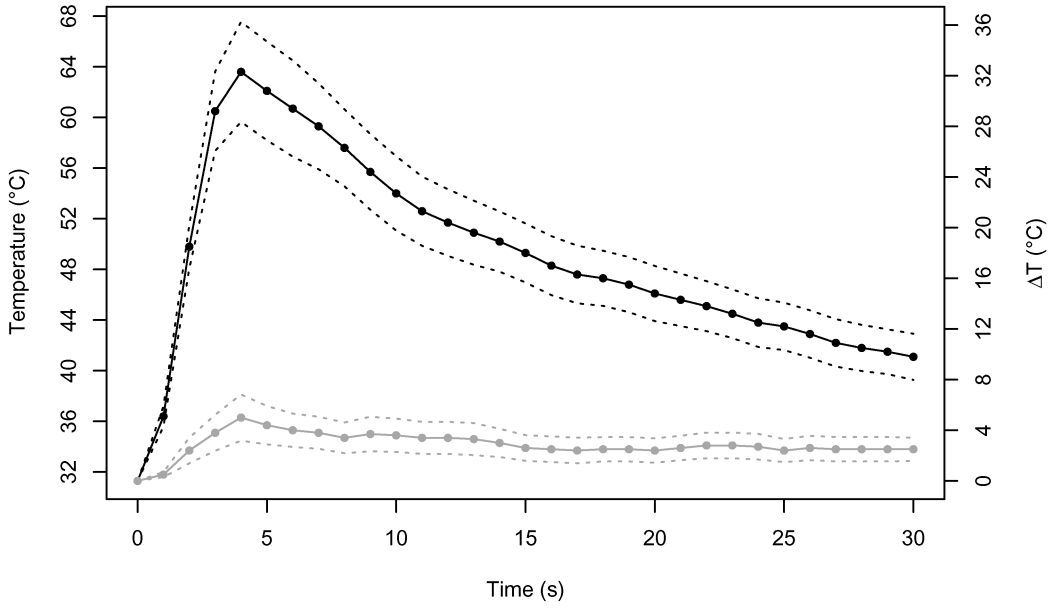

Figure 10. Mean temperature (left y-axis label) and mean temperature rise DT (right y-axis label) over time with 95% confidence intervals (dashed lines) at 4.5 mm (black dots) and 11.5 mm (grey dots) from the edge of the coagulation site.

3.4.3. Tissue surface temperature

The profile of surface temperature versus time and distance from the coagulation site is depicted in Figure 11. Mean initial temperature (± SD; range) was 27.9°C (± 2.2°C; 24.0–32.0°C). The curves demonstrate that the tissue

closest to the coagulations site heated up first. Immediately after coagulation (10 s), mean temperature at the coagulation site was approximately 85°C and did not drop below 50°C for the next 20 seconds.

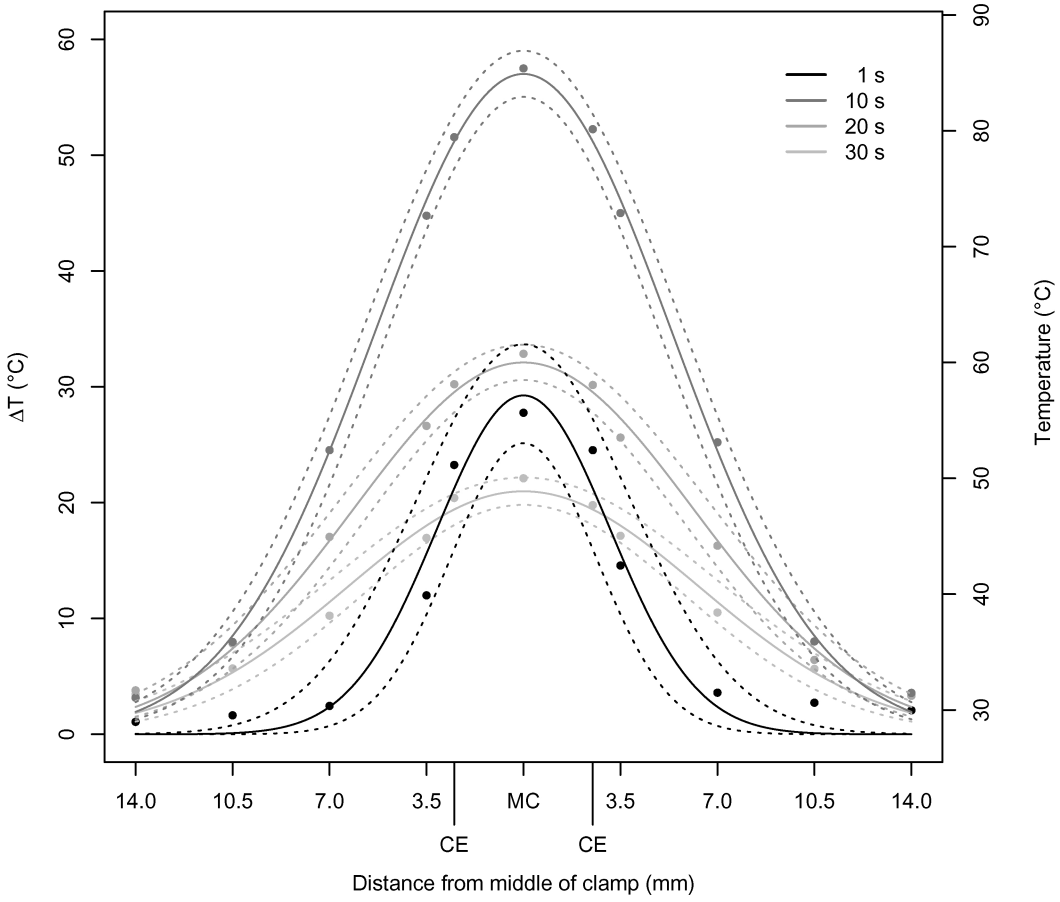

Figure 11. Distribution of mean surface temperature rise as a function of distance from the coagulation site, shown as data points (dots) and fitted curves (solid lines) with 95% confidence interval curves (dotted lines). MC = middle of clamp, CE = edge of clamp (= 2.5 mm from the middle of the clamp). Points and curves represent data at 1, 10, 20 and 30 seconds (black, dark grey, grey, and light grey, respectively).

3.4.4. Information value of thermal probe data vs. thermal camera measurements

The thermal probe and thermal imaging methods were compared for the temperature data measured at 4.5 and 10.5 mm from the edge of coagulation site at t = 1, 10, 20 and 30 seconds using the statistical methods described by Bland and Altman [47]. Differences for mean values were increased, while relative differences remained approximately constant. For this reason, the Bland-Altman plot in Figure 12 shows log transformed data.

On back transformation the temperature values recorded with the thermal camera were, on average, 102% of those obtained with the thermal probes (95% confidence interval of mean [99%; 105%]).

Thus, camera-recorded temperatures were 2% higher on average, corresponding to an average 1°C difference between thermal camera and thermal probe measurements. However, the limits of agreement indicated that 95% of the camera-recorded temperatures could be expected to lie in an interval between 66% and > 158% of the temperatures measured with the thermal probes, corresponding to a difference range between −12°C and +18°C for the mean observed temperature values.

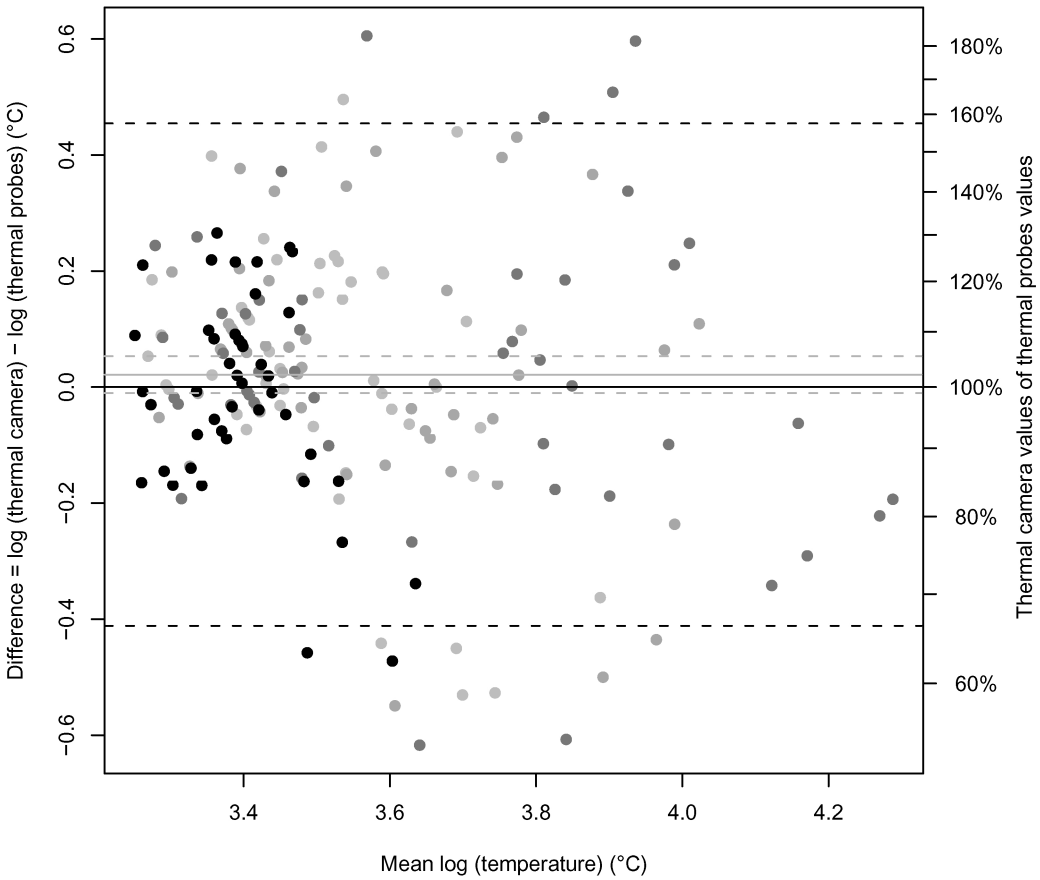

Figure 12. Bland-Altman plot of log transformed temperature data recorded with a thermal camera and with thermal probes. Differences at 1, 10, 20 and 30 s are plotted as dots (black to lightgrey). Also shown are the mean difference of logs (grey solid line) with 95% confidence intervals (grey dashed lines) and limits of agreement (black dashed lines).

3.4.5. Histological analysis

A linear relationship was observed between internal and external LTD (R^2_{adj} = 0.82). The external LTD was 1.5 times greater than the internal LTD. All analyses reported below are based on mean LTD (mLTD), which was 2.34 mm (± 0.49 mm; range 1.6–3.4 mm).

The scores for epithelial alteration and tubal architecture were strongly correlated with mLTD (both r_s = 0.70), presence of tissue fragmentation was weakly correlated with mLTD (r_s = 0.22), and basophilia was moderately correlated with mLTD (r_s = 0.59). The sum score (Smi) for all 4 criteria was strongly correlated with mLTD (r_s = 0.82). Further analysis led to a simplified score (sSmi), the sum of epithelial alteration and tubal architecture, with r_s = 0.81, a correlation of similar strength as the one between Smi and mLTD.

3.4.6. Macroscopic analysis

Macroscopic criteria were also compared with the mLTD. The scores for tissue clarity and tissue desiccation were strongly correlated with mLDT (r_s = 0.76 and r_s = 0.82, respectively) while tissue charring and instrument sticking were both weakly correlated with mLTD (r_s = 0.29 and r_s = −0.27, respectively). The sum score (Sma) of all 4 criteria was strongly correlated with mLTD (r_s = 0.81). The best simplified score (sSma) was found to be the sum of the scores for tissue clarity and tissue desiccation with a correlation coefficient of r_s = 0.83, which was as strong as correlation between Sma and mLTD.

4. DISCUSSION

4.1. General Problem Statement

Ever increasing numbers of minimally-invasive laparoscopic procedures have been introduced into surgical routine [2, 3] with clear clinical benefits. This success has largely depended upon continuous improvements in technology, with the introduction of electrosurgical vessel sealing probably being one of the most fundamental [2, 5, 6].

However, the introduction of energy-based vessel ligation has also been associated with a number of complications. This has been particularly true for more complex laparoscopic interventions [17, 18]. One of the main concerns is the quality of vessel sealing, which is often only suboptimal [3, 7, 15]. Another concern is the occurrence of thermal injuries and ischemic injuries that occur because of direct thermal application or thermal spread to adjacent tissues. These thermal injuries can lead to severe complications and often go unnoticed during surgery [2, 3, 7, 19, 39, 48].

Yet another concern is the adhesiogenic potential of electrocoagulation, since it is known to predispose to adhesion formation but is widely used in surgery [21, 22]. Thus, there is a definite need for further experimental and clinical studies to better understand the biothermomechanics of thermal fusion and its risks. Many factors in the application of electrosurgery are based on surgeon's preferences and anecdotal data as opposed to objective data.

Therefore our objectives in the underlying studies were:

- to develop and test a new intelligent bipolar vessel sealing mode to improve the strength and reliability of the vessel seals in an in vitro porcine model

- analyse the biothermodynamics behind bipolar vessel sealing in order to better understand the process of thermal fusion in an in vitro poricine model
- investigate the relationship between electrocoagulation and adhesion formation in an in vivo rodent model
- and to develop a human in-vivo in-situ model for analyzing the basic mechanisms of thermal spread and thermal tissue damage and their extent.

4.2. Discussion Study 1: Intelligent, impedance-regulated, pulsed coagulation in a porcine renal artery model

4.2.1. Problem Statement

The widespread use of heating tissue to achieve coagulation is primarily driven by the availability of and improvement in technologies that perform this efficiently, but not by a detailed understanding of the biothermomechanics of the process [49]. Improvements in instrumentation and technology have made a significant contribution to the consistent advances in laparoscopic surgery. Every improvement, however, generates new complications, and the introduction of energy-based vessel ligation has been no exception.

Thermal fusion is influenced by the amount of heat input over time and the length of time the heat is applied. In bipolar coagulation, the interaction between impedance and current creates the high temperatures necessary for vessel coagulation, and the change in tissue impedance indirectly indicates when this temperature has been reached.

Pulsed coagulation appears to generate vapor zones with high impedance during the pulse. Seeking the path of lowest impedance, the current generates a high-energy density around these zones, leading to additional thermal effects. During the pause, while the forceps and the tissue are cooling, the vapor

condenses and the moisture returns. During subsequent pulses, this process is repeated until uniform coagulation is achieved [5, 9]. Denaturation of collagen is enhanced through hydration by decreasing its stability [49], therefore continuous hydration during pulsed coagulation may very well lead to increased seal quality [5, 9].

Vessels can be successfully coagulated with CPC [5, 8, 15], but until now, most pulsed bipolar coagulation methods in current use are based on a predetermined pulse frequency with fixed bursts and pauses. They are dependent on impedance but do not regulate themselves on it. Due to the variability of vascular tissue and vessel size, this may result in overcoagulation or inadequate ligation, which both lead to seal failure.

Conventional coagulation can take up to 12 seconds, and in our study, resulted in seal failures in a high number of cases. We suggest that this is primarily due to the long fraction of the pulse during which the current decreases and the energy density is reduced. This fraction increases as the coagulation process advances, whilst the pulse and pause intervals remain constant. The result is that the overall current and energy density is too low to ensure the high temperature and rapid increase in temperature required for thermal fusion.

To overcome this limitation, we developed a new modulation of the alternating current required, aiming to achieve ‘intelligent’ pulsed bipolar coagulation (IPC) with a dynamic modulation process, where the duration of pulses and pulse-pause sequences adapts itself to increasing tissue impedance. The adaptive initiation of subsequent pulses leads to a higher energy input per time unit and a more rapid rise in temperature, leaving the tissue to cool only during the pause designed for this very purpose. The present study evaluated the newly developed modulation of our coagulation software in an isolated porcine renal artery model.

4.2.2. Interpretation of results

Other reports have shown that arteries with a diameter of more than 5 mm show a higher rate of seal failures [15]. In the present study, analysis of the relationship between vessel size and burst pressure confirmed that the seal quality was better the smaller the vessel, suggesting that the burst pressure decreases reciprocally with an increase in vessel diameter. Our results further show that IPC led to successful seals in 95% of vessels and only 66% after CPC-I and 61% after CPC-II.

The number of seal failures in our study was higher than in other studies with 75–95% successful seals [5, 7, 8]. This is probably because we used a very strict definition for failure (burst pressure lower than 200 mm Hg), as we felt that a rigorous approach is required when testing new technology. Moreover, in this study, vessels with relatively large diameters were studied and all failures, including technical failures, were included in the analysis, which will also have contributed to the higher number of failures.

Multiple CPC in our experimental setting not only took longer but did not lead to a superior sealing quality than was achieved after single coagulation with IPC. It therefore appears that multiple coagulation is not superior to IPC, may cause more lateral thermal damage, and may carry a higher risk of rupture. However, since no prior reports on consecutive coagulation of vessels under experimental conditions were found in the literature, and given the small number of vessels in this part of our study, the implications of these results should be viewed with caution.

4.2.3. Critics

One limitation of our study is that despite randomization, the operator was aware of the coagulation technique in use, thus introducing potential bias. However, most parameters that could potentially be influenced, such as the

pressure applied and the duration of the sealing process were standardized and the different modes were used according to a randomized scheme. Only after preparing the vessel and before the sealing process did the operator change the mode according to the randomized study protocol. Another limitation of our study is the lack of follow-up data inherent in the model chosen. Also, the dissection of the arteries might have caused damage to the vessels tested. However, this seems unlikely, because all due care was taken.

Unlike other study groups, we used bloodless coagulation in our experiments. To study the effect of the different current modulations on the vessel wall and the ensuing seal with only a minimum of variables, all blood was flushed from the vessel before testing. We feel that isolated preserved blood would not ideally mimic the clinical situation, and that the specific effect of blood perfusion on the process of coagulation can only be determined in vivo.

4.2.4. Conclusions

In conclusion, this study in a porcine renal artery model demonstrates that a new, intelligent, impedance-regulated modulation of energy-based vessel coagulation appears to achieve safer thermal fusion of vascular tissue than commonly used methods in this specific setting. Unlike CPC, in which the coagulation process is preset by the operator, with IPC, the current flow is regulated by the impedance feedback from the tissue being coagulated.

This promising technique requires further investigation in vivo, including long-term analyses. We found that single application of our newly developed technique was significantly better than the coagulation methods in conventional use. Nonetheless, these results should be interpreted with caution until the results of in vivo follow-up studies are available.

4.3. Discussion Study 2: Thermal conduction, compression and electrical current – an evaluation of major parameters of electrosurgical vessel sealing in a porcine in-vitro model

4.3.1. Problem Statement

Specific biothermomechanical parameters such as compressive pressure and coagulation temperature are believed to be key to the success of electrosurgical vessel sealing. Moreover, the role of high-frequency electrical current as opposed to pure thermal fusion is unclear. Yet, little is known of these parameters and only very few analyses of these parameters are available today. Thus, the current study was designed to investigate the influence of compressive pressure, temperature, tissue shrinkage and electrical current versus thermal conduction on successful vascular sealing.

4.3.2. Interpretation of results

The calibres of the sealed porcine vessels (arteries 5.1 mm and veins 4,8 mm) were comparable to the human uterine artery, which ranges from 3-5 mm in diameter [50].

The amount of compressive pressure during the coaptation process significantly influenced the quality of the achieved seal. In arteries especially, too low a CP led to a high number of seal failures. Increasing CP resulted in a greater number of successful seals and a higher mean burst pressure. This shows that there is a minimum threshold CP below which coaptation cannot be guaranteed. On the other hand, increasing CP excessively also reduced the seal quality. This shows that there is also an upper limitation to the CP, above which the vessel wall is damaged in such a way that optimal coaptation can no longer be achieved.

Consequently there is a limited compression pressure interval for safe vessel sealing. For arteries, this interval lies around 270 mN/mm². In contrast, the optimal pressure for veins lies around 200 mN/mm². This may reflect their lower wall thickness.

In successful arterial seals, the maximum temperature during coaptation was significantly higher than in seal failures. This indicates that there is a positive correlation between temperature and seal stability. In veins, however, the situation was reversed with the maximum temperature for successful seals significantly lower than for seal failures. These findings indicate that, similar to the situation for CP, there is a specific optimal temperature for bipolar coaptation. The optimal temperature appears to depend on the thermal capacity of the vessel, as determined by its wall thickness, and this may account for the differences between arteries and veins.

Another important consideration is that vessels shrink in response to thermal fusion and water vaporization. The difference in longitudinal arterial tension before and after coaptation (ΔlT) differed significantly between successful seals versus seal failures. This indicates that vessel contraction correlates with the strength of the seal and therefore represents an indirect indicator of coaptation success.

In principle, it is possible to seal both arteries and veins through purely thermal conduction without application of an electrical current. The effectiveness of this method was only 19.2% for arteries and 52.4% for veins, which is considerably less than what was achieved by bipolar electro-coaptation. The amount and rate of heat deposition, coupled with the properties of the sealed vessel govern the resulting changes at the microscopic and macroscopic scale [43].

Coaptation by purely thermal conduction dries the vessel wall from the outside to the inside and consequently results in a dry outer layer around the still moist inner layers with a differential heat distribution over the cross-section of the

vessel wall. This differential in cross-sectional heat distribution would increase with thermal capacity of the vessel wall and temperature. As a result, it is particularly great for the thick-walled arteries leading to inadequate results.

In contrast, bipolar electro-coaptation heats the vessel layers simultaneously through their impedance to the electrical current rather than by thermal conduction from the outside. The instant dissipation of energy into the tissue by electro-coaptation compares favourably with the relatively slow dissipation of energy by thermal conduction. In the light of our presented data and these theoretical considerations it is not efficient to use coaptation by thermal conduction in lieu of electro-coagulation in a clinical setting.

4.3.3. Critics

The current study has three limitations, which represent directions for future study. Firstly, the results should be validated in the presence of blood in vivo. Secondly, the number of vessels was rather small in this pilot study and should be increased in further studies. Thirdly, the integral of the temperature-time curve should be determined in addition to mT. This will shed light on time as a factor in electrosurgical vessel sealing. Moreover, the presented results necessitate further analysis of compressive pressure and temperature in instruments which are already in clinical use. Data on these matters is scarce but necessary as the basis for future instrument designs.

4.3.4. Conclusions

In summery, the current investigation defines the optimal compressive pressure interval during vessel sealing for arteries and veins. We also demonstrate an association between temperature, bipolar high-frequency effects and successful sealing. These findings have implications for the rational design of future electrosurgical instruments.

4.4. Discussion Study 3: The extent of adhesion induction through electrocoagulation and suturing in an experimental rat study

4.4.1. Problem Statement

Bipolar coagulation is routinely used in open and laparoscopic surgery. However, it is believed to predispose to adhesion formation. The current study investigates the extent of adhesion induction through electrocoagulation, suturing and mechanical trauma of the peritoneum and the abdominal wall in an experimental rodent animal model.

4.4.2. Interpretation of results

In our study, mechanical denuding of the peritoneum without damage to the underlying musculature caused no adhesion formation. After minimal electrocoagulation, adhesion incidence of the traumatised area was only 14% whereas after extensive electrocoagulation, minimal electrocoagulation with suturing or mechanical denuding with suturing the adhesion incidence was 100%. This is despite the narrower traumatised areas in the sutured groups, which was chosen to allow suturing over the whole defect [40].

As for adhesion quantity, there was minimal quantity with minimal electrocoagulation but ample quantity for all other modalities except for denuding without surgery which resulted in no adhesion formation at all.

In this study, trauma by electrocoagulation only, resulted in dense adhesions only whereas when either coagulation or denuding was combined with suturing of the underlying musculature both filmy and dense adhesions were found. Further investigation is needed to determine whether the mode or the extent of traumatisation correlates with adhesion quality especially since the clinical relevance of adhesion quality (filmy versus dense) is not yet clear.

Electrocoagulation significantly differs from other modalities used in experimental models with regards to the quality of the injury produced. On the one hand, the thermal spread leads to damage to deeper structures. On the other hand, electrocoagulation leads to sealing of blood vessels which is not a feature of mechanical injury by abrasion with a brush or excision of the peritoneum. In spite of this, we found no statistically significant difference in adhesion formation between mechanical removal of the peritoneum and peritoneal destruction by minimal electrocoagulation.

These study's results suggest that superficial trauma, either by mechanical denuding or minimal electrocoagulation do not necessarily lead to adhesion formation. However, trauma of the layers deep to the peritoneum – either by more severe electrocoagulation or by additional suturing through the underlying musculature may lead to increased adhesion formation. This highlights the role of the tissue and musculature underlying the peritoneum in adhesion formation and suggests that the additive effect of suturing on adhesion formation, which is well established for mechanical traumatisation of the peritoneum [32] also exists for trauma by electrocoagulation in this model.

Finally, the results of the current investigation suggest that the effect of bipolar electrocoagulation on adhesion formation might depend on the extent of coagulation. There appears to be a spectrum concerning the depth of trauma by electrocoagulation at the lower end of which there is little adhesion formation and the higher end of which there is extensive adhesion formation.

4.4.3. Critics

A number of limitations to which every experimental study is prone, should be taken into consideration when interpreting the current results. Firstly, for the limited number of animals, the results of this study need to be verified in a larger study.

Secondly, different areas were traumatized in different groups. However, with respect to the results, we feel confident to compare the groups and draw conclusions from that. In fact, the increased adhesion formation despite the smaller traumatised areas serves to highlight the important additive effect of suturing. Our results are unambiguous in demonstrating that minimal coagulation plus suturing leads to significantly more adhesions than miminal coagulation alone in this model.

Thirdly, the current investigagion only considers the parietal peritoneum. These results need to be replicated for the visceral peritoneum and other tissues commonly traumatised during surgery, such as the ovary.

Finally, in this study, we took no specific action to keep the tissue moist during the intervention although tissue dessication may be one of the factors that leads to adhesion formation. Since the overall duration of the surgery was approximately 20 minutes and the differences in the duration between the groups were negligible, we are confident that tissue dessication was no confounding factor in this experiment. In future experiments tissue dessication could be standardised between the groups by waiting a constant time before closure of the abdominal cavity.

4.4.4. Conclusions

In conclusion, superficial trauma mostly limited to the parietal peritoneum may be a neglible factor in adhesion formation. This appears to be irrespective of the mode of trauma. However, additional trauma of the underlying tissues, either by deeper electrocoagulation or suturing, lead to significantly increased adhesion formation. These data also show that there is a spectrum concerning the extent of trauma by electrocoagulation at the lower end of which there is little adhesion formation.

4.5. Discussion Study 4: Quantifying electrocoagulation-induced thermal effects and damage to human tissue: An exploratory study using the fallopian tube as a novel in-vivo in-situ model

4.5.1. Problem Statement

The objective of the present study was to establish an in-vivo in-situ model to study the thermal effects of electrocoagulation on human tissue and the damage it induces. There is a definite need for a standardized model, given the multitude of available electrosurgical devices, the unacceptably high number of thermal complications, and our deficits in understanding in detail the biothermomechanics of thermal fusion.

4.5.2. Interpretation of results

The ultimate endpoint of thermal damage is tissue necrosis. Histologically, thermal damage has always been described in a mostly dichotomous fashion and its detection and evaluation has reportedly been difficult [51, 52]. To date, very few quantitative approaches have been pursued to evaluate thermal damage in a standardized yet detailed fashion [53]. However, these were developed for skin lesions and were not suitable for our purposes. In the present study, we defined as our gold standard the assessment of mLTD as revealed by LDH staining according to Sherwood and Flotte [42]. This method enables the detection of oxidative enzymes that correlate with cell function and activity and therefore is particularly suited to the assessment of thermally damaged tissue.

In our study, lateral thermal damage to fallopian tube tissue was very strongly correlated with the maximum temperature rise within the tissue, which is in accordance with theoretical models of thermal fusion [54] and aspects supporting LDH staining. Both parameters demonstrated a moderate negative correlation with tubal caliber, which suggests that heat development decreases

with increasing tissue volume, thus limiting heat development and hence, to a certain degree, thermal damage.

Interestingly, the thermal damage measured on the surface of the fallopian tube was approximately 1.5 times higher than at the center of the tube, indicating that the lateral spread of the thermal lesion was greater than its depth of penetration. One explanation for this phenomenon could be that the fluids on the surface and in the outer layers of the tissue heat up and evaporate more quickly than those in the deeper layers, thus adding an exogenous heat component to the endogenous heat effect through the release of energy as dictated by Joule's Law.

In electrocoagulation, tissue response is governed by the amount of heat and the dynamics of heat deposition, in conjunction with the tissue properties and mechanical parameters [37, 43]. Thus, temperature is a key factor in thermal fusion as well as thermal damage. In our study, mean surface temperature at the coagulation site was approximately 85°C immediately after coagulation and did not drop below 50°C during the next 20 seconds.

Mean deep-tissue temperatures at 4.5 mm lateral to the coagulation site did not exceed 50°C for more than 10 seconds. The resulting mean LTD measured 2.3 mm, which is consistent with literature reports that the minimum temperature for tissue coagulation is 50°C [55, 56] and that thermotherapy requires temperatures of about 50°C or 55°C to be maintained for at least 15 seconds [55, 57].

Both thermal probes and thermal cameras generate valuable information on the changes in deep-tissue and surface temperature in space and time. However, the data obtained with these two modalities differ considerably. A small portion of the differences can be explained by the observation that the spread of thermal damage is greater on the surface than within the tissue because the heating process produces higher temperatures on the tissue surface. This

interpretation is supported by our finding that mean temperatures were 1°C higher for the thermal camera compared with the thermal probes. Until such time as this has been studied in greater detail, it seems best to combine the two modalities to obtain comprehensive and accurate temperature data.

Based on previous reports of thermal artifacts in the fallopian tube and the cervix [43-45], we devised a histological score Smi consisting of the four criteria epithelial alteration, tissue fragmentation, tubal architecture and basophilia. In our present study, Smi was strongly correlated with mLTD. Therefore, our composite score and its individual criteria were, in combination with LTD detection, suited to assessing the effects of tissue coagulation in a more quantitative manner, thus rendering them more comparable.

Detailed, reliable histological evaluation is particularly important in this model since lateral spread as assessed by histology is less pronounced than indicated by real-time thermography. This is because the extent of permanent damage depends not only on maximum temperature but also on the duration of heat application [38]. Moreover, our score could be useful as a tool to compare electrosurgery with other modalities with regard to collateral damage, e.g., ultrasonically induced proximity damage, which is not macroscopically detectable [58]. Although it appears that a simplified score, sSmi, comprising only two criteria – epithelial alteration and loss of histological tubal architecture – adequately reflects the induced histological changes, further studies will be needed to confirm or refute this finding.

In clinical routine, thermal effects and even injury are often only assessed macroscopically. As with histological evaluation, there is no established standardized quantitative protocol. We therefore considered it necessary to include macroscopic analysis in our model as a 4^{th} category. The criteria constituting the macroscopic score were available from the literature [36] but had not been evaluated with regard to their ability to reflect tissue damage as defined by histology. In our present study, the sum score, Sma, was found to

reflect the histology of our specimens quite accurately. The simplified score, sSma, representing the sum of only the two strongly correlated criteria, produced equally good results and can be considered sufficient for macroscopic analyses.

4.5.3. Critics

Our model as reported here admittedly leaves room for improvement. Firstly, maximum temperature rise as suggested by the thermal probe data was reached after about 4 seconds, a time at which no thermal images were taken. Secondly, all thermal images were taken on verbal command, which may have led to some delay. These issues will be resolved in future studies by using automated thermal imaging at a rate of 1 Hz. Moreover, the innermost thermal probes will be positioned much closer (2 mm instead of 4.5 mm) to edge of the clamp than in the present study so as to capture the details of the temperature dynamics more accurately and avoid missing most of the temperature dynamics. Finally, the exact position of the camera is a compromise between adequate spatial resolution and obstruction of the surgeon's view and range of action. The low standard deviations of our measurements, however, demonstrate that the task is feasible and reproducible.

4.5.4. Conclusions

In summary, we consider that our model produces comprehensive and easily reproducible data in a standardized manner suitable for the evaluation of thermal effects and damage to human tissue. In combination, the tissue surface and deep tissue temperature measurements enable a more in-depth analysis of tissue temperature profiles and their dynamics than either method by itself. Quantitative macroscopic and microscopic evaluations of thermal damage based on multiple criteria allow the thermal effects of various instruments to be compared at different coagulation settings. Consolidated evaluation of

temperature and thermal damage enables investigators to better understand, and also quantify, the relationship between heat and tissue damage.

For the first time, in-vivo in-situ real-time temperature measurements in humans have been combined with thermography, macroscopic analysis, and histology to establish a standardized model for further investigation of the thermal effects and damage induced by electrocoagulation. Understanding the underlying biothermomechanics will help to develop safety guidelines for the handling of electrosurgical instruments in laparoscopic surgery.

5. SUMMARY

Objective:

Bipolar vessel sealing and electrosurgery in general is pivotal in surgical and especially minimally-invasive surgical hemostasis. However, quality of vessel sealing is only suboptimal and major coaptive desiccation parameters have yet to be investigated in depth. Moreover, the potentially hazardous capacity of electrosurgery to induce both post-operative adhesions and thermal complications such as tissue necrosis has not been looked into in detail hitherto.

In order to (1) optimize bipolar vessel sealing, to (2) better understand the biothermomechanics of thermal fusion, to (3) analyze the relationship between electrocoagulation and adhesion formation and to (4) develop a human in-vivo in-situ model for quantifying electrosurgery-induced thermal tissue effects and thermal tissue damage, the following studies were conducted.

Methods and Results:

Ad (1): In a prospective, randomized experimental study in an academic research environment, the efficacy of conventional pulsed coagulation (CPC) and newly developed intelligent, impedance-regulated, pulsed coagulation (IPC) was compared in the sealing of porcine renal arteries from female Swabian pigs. Renal arteries were harvested, flushed with saline and sealed with bipolar open forceps using high-frequency modulations of CPC (CPC-I: 800 ms pulse/30 ms pause; CPC-II: 800 ms pulse/300 ms pause) or IPC (self-regulation of the current flow to tissue impedance during thermal alteration). Additional vessels underwent multiple CPC. Burst pressure and seal failure were measured by increasing the pressure in the sealed arteries with saline infusion until rupture of the seal or the vessel wall. The main outcome measures were the mean burst pressure, number of instant and secondary seal failures, and relation of burst pressure to vessel diameter.

It was found that mean burst pressure after IPC (585.5 ± 56.8 mmHg) was significantly higher than that after CPC (CPC-I: 372.6 ± 40.0 mmHg; CPC-II: 334.2 ± 44.2 mmHg) ($p<0.05$). Only 5.0% of the vessel seals after IPC, but 34.0% and 39.5% after CPC-I and CPC-II showed instant or secondary seal failures ($p<0.05$). Seal quality after multiple CPC was comparable to that observed after the single IPC application (burst pressure: 597.3 ± 60.1 [MCPC-I] and 656.2 ± 56.5 mmHg [MCPC-II]; seal failure: 0%).

Ad (2): In a randomized, controlled experimental trial, the impact of compressive pressure, thermal conduction and electrical current effects on seal quality were investigated in a porcine in-vitro model of vessel sealing in an academic research environment. 106 porcine vessels were sealed with either bipolar current or thermal conduction. Main outcome measures were compressive pressure on the coagulation site and maximum temperature were varied and monitored. Additionally, the longitudinal vessel tension was measured. The burst pressure of the resulting seal was determined as an indicator of seal quality.

We found that in bipolar coaptation, seal quality depends on the compressive pressure applied to the coagulation site in both arteries and veins. The optimal pressure interval was around 270mN/mm² for arteries and 200mN/mm² for veins. Deviation from these optimal pressures towards low and high extremes led to significantly fewer successful seals. We also found that both maximum coaptation temperature and vessel shrinking correlated with the seal quality. This correlation was reciprocal in arteries and veins. Thermal conduction alone was significantly less successful than sealing by bipolar current.

Ad (3): In a randomized, controlled experimental trial, the effects of three types of peritoneal trauma occurring during surgery (high-frequency bipolar current, suturing and mechanical damage) on post-operative adhesion formation were investigated in a rodent animal model in an academic research environment. In 35 female Wistar rats bilateral experimental lesions were created on the

abdominal wall in every animal. The effect of minimal electrocoagulation was examined by creating lesions (n = 14) through sweeps of a bipolar forceps with a duration of 1 second and standardized pressure. For extensive electrocoagulation standardised lesions (n=14) were created using sweeps of a duration of 3 seconds and 3 times greater pressure. For mechanical trauma, standardized lesions (n=14) were created by denuding the peritoneum mechanically. To study the additive effect of suturing, experimental lesions were created by suturing plus minimal electrocoagulation (n=14) or mechanical denuding (n=14). Main outcome measures were adhesion incidence, quantity and quality of the resulting adhesions were scored 14 days post-operatively. Adhesions were studied histopathologically.

We established that mechanical denuding of the peritoneum did not result in adhesion formation. After minimal electrocoagulation, mean adhesion quantity of the traumatised area averaged 0%. This contrasted with extensive electrocoagulation, where there was 50% adhesion quantity. Additional suturing increased mean adhesion quantity to 73% and 64% for superficial electrocoagulation and mechanical denuding respectively.

Ad (4): In a prospective, open, uncontrolled, non-randomized, single-center exploratory study, a human in-vivo in-situ model for analyzing the basic mechanisms of thermal spread and thermal tissue damage and their extent was developed. Unilateral fallopian tube coagulation (10 seconds) using a laparoscopic bipolar clamp at routine settings was conducted in eighteen adult patients undergoing open abdominal hysterectomy for benign disease. At this, deep tissue temperature (thermal probe), tissue surface temperature (thermal camera) were studied, and gross and histopathological lesions were assessed using a new, purpose-designed scoring system.

It was determined that lateral thermal damage (LTD; determined by lactate dehydrogenase staining), was strongly correlated with maximum coagulation temperature. Deep tissue LTD and surface LTD were linearly related.

Histopathological and macroscopic criteria for thermal effects and damage and the corresponding scores proved functional and strongly correlated with LTD. Measurement of deep tissue and tissue surface temperatures consistently yielded complete temporal and spatial temperature distributions that were describable by the heat equation.

Conclusions:

It was found that in an isolated porcine renal artery model, self-regulating modulation of energy-based vessel coagulation achieved superior thermal fusion of vascular tissue than CPC. This promising novel technique should therefore be further analyzed to determine its in-vivo efficacy in long-term studies. Moreover it was ascertained that compressive pressure during coaptation determines the seal quality. Upper and lower pressure boundaries for safe coaptation exist for both arteries and veins. Vessel sealing by thermal conduction without electrical current effects is possible but represents a less effective method for coaptation. These findings have implications for the rational design of new electrosurgical instruments.

With regards to the adhesiogenic potential of bipolar tissue desiccation, we conclude that superficial trauma limited mostly to the parietal peritoneum may be a neglible factor in adhesion formation in this model. This appears to be irrespective of the mode of trauma. However, additional trauma to the underlying tissues, either by deeper electrocoagulation or suturing, lead to significantly increased adhesion formation. These data also show that there is a spectrum of electrocoagulation trauma at the lower end of which there is little adhesion formation.

Finally, the new purpose-designed in-vivo in-situ model allows standardized, reproducible, quantitative assessment of electrocoagulation-induced thermal effects and damage in human tissue. It will likely provide further insight into the underlying biothermomechanics and may prove useful in the development of safety guidelines for laparoscopic electrosurgery.

6. ACKNOWLEDGEMENTS

I would like to heartily thank Prof. Planck for the many helpful suggestions and the support with the interpretation of the results. I greatly thank Prof. Isaacson for the encouragement and mentorship over the years. To my collaborators in the electrosurgery and adhesion prophylaxis research groups, namely Dr. Sara Brucker, Dr. Wolfgang Zubke, Dr. Bernhard Kraemer and Dr. Taufiek Rajab, I am grateful for the continuous support.

"Without friends, no one person would choose to live, though he had all other goods (Aristotle, 384 BC – 322 BC)". Thus, special thanks to my friends for all the support they have lent me over all these years.

And finally, my very special thanks to my family and in particular my wife Lisa-Maria, my daughter Rosalie, my father Diethelm, my mother Gabriele and my brother Markus, whom I owe everything I am today. Their profound friendship, great enthusiasm and zeal together with their confidence and faith in me are what have shaped me to be the person I am today.

- "Intelligent, impedance-regulated, pulsed coagulation in a porcine renal artery model"
  I wish to thank Ralf Klein for technical support and Alistair Reeves for editorial assistance.

- "Thermal conduction, compression and electrical current – an evaluation of major parameters of electrosurgical vessel sealing in a porcine in-vitro model"
  I wish to thank Dipl.-Ing. Sven Egner and Dipl.-Ing. Christian Seier for technical assistance and Dr. Ulf Ellwanger and Dr. Holger Lüdtke for support with the statistical analysis.

- Quantifying electrocoagulation-induced thermal effects and damage to human tissue: An exploratory study using the fallopian tube as a novel in-vivo in-situ model

  I wish to thank Dr. Peter Fritz and Dr. Thomas J. Flotte for histological analysis, Dr. Markus Enderle and Dipl.-Ing. Daniel Schäller for technical assistance and Dr. Birgitt Schönfisch for support with the statistical analysis.

7. PRESENTATIONS, PUBLICATIONS AND PRIZES

- "Intelligent, impedance-regulated, pulsed coagulation in a porcine renal artery model" (Fertil Steril. 2007 Jul;88(1):206-11. Epub 2007 Apr 26. (Impact Factor3.3))
    - **Poster Presentation** at the 33rd Annual Meeting of the American Association of Gynecological Laparoscopists (AAGL) in San Francisco, California, USA (2004); **Best Scientific Poster Award** from the American Association of Gynecological Laparoscopists (AAGL) at the 33rd Annual Meeting
    - **Oral Presentation** at the 13th Annual Congress of the International Society for Gynecological Surgery (ISGE) in London, UK (2005)

- "Thermal conduction, compression and electrical current – an evaluation of major parameters of electrosurgical vessel sealing in a porcine in-vitro model" (J Minim Invasive Gynecol. 2008 Sep-Oct;15(5):605-10. Epub 2008 Jul 21. (Impact Factor 1.8))
    - **Oral Presentation** at the 36th Annual Meeting of the American Association of Gynecological Laparoscopists in Washington, D.C., USA (2007)
    - **Oral Presentation** at the 16th Annual Congress of the European Society of Gynecological Endoscopy (ESGE), in Portoroz, Slowenia (2007)

- "The extent of adhesion induction through electrocoagulation and suturing in an experimental rat study" (Fertil Steril. (Impact Factor 3.3) in press (accepted Dec 2008))
    - **Oral Presentation** at the 15th Annual Congress of the European Society of Gynecological Endoscopy (ESGE) in Strasbourg, France (2006)

- **Poster Presentation** at the 17th Annual Congress of the European Society of Gynecological Endoscopy (ESGE) in Amsterdam, Netherlands (2008); **Best Scientific Poster Award** from the European Society of Gynecological Endoscopy (ESGE) at the 17th Annual Congress
- **Oral Presentation** at the 37th Annual Meeting of the American Society of Gynecological Laparoscopists (AAGL) in Las Vegas, USA (2008)

- "Quantifying electrocoagulation-induced thermal effects and damage to human tissue: An exploratory study using the fallopian tube as a novel in-vivo in-situ model"
  - **Oral Presentation** at the 36th Annual Meeting of the American Society of Gynecological Laparoscopists (AAGL) in Washington, USA (2007)
  - **Oral Presentation** at the 16th Annual Congress of the European Society of Gynecological Endoscopy (ESGE), in Portoroz, Slowenia (2007)

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9. CURRICULUM VITAE

ANGABEN ZUR PERSON

Geboren:	25. Juni 1983 in Homburg/Saar
Nationalität:	Deutsch
Familienstand:	verheiratet mit Lisa-Maria Wallwiener, geborene Roederstein Tochter Rosalie Elli Marie (geboren am 24.02.2008)

AUSBILDUNG

24.06.2009	Ärztliche Prüfung (2. Staatsexamen)
02/08 – 02/09	Medizinstudent im Praktischen Jahr (Studienjahr 6) Schwerpunkte: Allgemeine und Plastisch-Rekonstruktive Chirurgie, Gynäkologie und Geburtshilfe sowie Innere Medizin - Harvard Medical School, Mass. General Hospital, Boston, USA - Oxford University, John Radcliffe Hospital, Oxford, England - Cornell University, New York Presbytarian Hospital and Sloane Kettering Memorial Hospital, New York, USA - Technische Universität München, Klinikum rechts der Isar, München
04/05 – 02/08	Medizinische Fakultät der Technischen Universität München: Klinischer Studienabschnitt
12/07	2. Amerikanisches Staatsexamen der Humanmedizin
05/07	1. Amerikanisches Staatsexamen der Humanmedizin
03/05	Physikum (97. Perzentil deutschlandweit)
04/03 – 02/05	Medizinische Fakultät der Ludwig-Maximilians-Universität: Vorklinischer Studienabschnitt
07/02 – 04/03	Fallschirmjäger und Kommando Spezialkräfte, Calw
09/98 – 06/02	Kepler Gymnasium Tübingen
09/93 – 07/98	English Institute Heidelberg (Gymnasium)
09/89 – 06/93	Grundschule Leimen

AUSZEICHNUNGEN UND STIPENDIEN

10/08	Award “Best Scientific Poster” der European Society of Gynecological Endoscopy (ESGE): “The extent of adhesion induction through electrocoagulation and suturing in an experimental rat study”
09/08	Award “Best Poster” der Deutschen Gesellschaft für Gynäkologie und Geburtshilfe (DGGG) in der Kategorie Onkologie: „Auf dem Weg zu einer automatisierten Studienrekrutierung in der Senologie – Erstellung eines Systems zur Abbildung von Studienkriterien“
03/08	Best Practice Award “Mehr Dialog für Krebs” der Deutschen Krebsgesellschaft für das Portal www.brustkrebs-studien.de
11/04	Award “Best Scientific Poster” der American Society of Gynecological Laparoscopists (AAGL)
02/04	Aufnahme in die Studienstiftung des deutschen Volkes

10.ADDENDUM

- “Intelligent, impedance-regulated, pulsed coagulation in a porcine renal artery model” (Fertil Steril. 2007 Jul;88(1):206-11. Epub 2007 Apr 26.
- “Thermal conduction, compression and electrical current – an evaluation of major parameters of electrosurgical vessel sealing in a porcine in-vitro model” (J Minim Invasive Gynecol. 2008 Sep-Oct;15(5):605-10. Epub 2008 Jul 21.
- “The extent of adhesion induction through electrocoagulation and suturing in an experimental rat study” (Fertil Steril. 2009 Jan 14. [Epub ahead of print])
- Manuscript under Review: “Quantifying electrocoagulation-induced thermal effects and damage to human tissue: An exploratory study using the fallopian tube as a novel in-vivo in-situ model”

Intelligent, impedance-regulated, pulsed coagulation in a porcine renal artery model

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Objective: To compare the efficacy of conventional pulsed coagulation (CPC) and newly developed intelligent, impedance-regulated, pulsed coagulation (IPC) in the sealing of porcine renal arteries.
Design: Prospective, randomized experimental study.
Setting: Isolated porcine artery model in an academic research environment.
Animal(s): Female Swabian Hall pigs.
Intervention(s): Renal arteries were harvested from Swabian pigs, flushed with saline, and sealed with bipolar open forceps by using high-frequency modulations of CPC (CPC-I: 800-ms pulse, 30-ms pause; CPC-II: 800-ms pulse, 300-ms pause) or IPC (self-regulation of the current flow to tissue impedance during thermal alteration). Additional vessels underwent multiple CPC. Burst pressure and seal failure were measured by increasing the pressure in the sealed arteries with saline infusion until rupture of the seal or the vessel wall.
Main Outcome Measure(s): Mean burst pressure, number of instant and secondary seal failures, and relation of burst pressure to vessel diameter.
Result(s): Mean burst pressure after IPC (585.5 ± 56.8 mm Hg) was statistically significantly higher than that after CPC (CPC-I: 372.6 ± 40.0 mm Hg; CPC-II: 334.2 ± 44.2 mm Hg). Only 5.0% of the vessel seals after IPC, but 34.0% and 39.5% after CPC-I and CPC-II, showed instant or secondary seal failures, which also was a statistically significant difference. Seal quality after multiple CPC was comparable to that observed after the single IPC application (burst pressure, 597.3 ± 60.1 [MCPC-I] mm Hg and 656.2 ± 56.5 mm Hg [MCPC-II]; seal failure rate, 0).
Conclusion(s): In an isolated porcine renal artery model, self-regulating modulation of energy-based vessel coagulation achieved superior thermal fusion of vascular tissue than did CPC. This promising novel technique should be analyzed further to determine its in vivo efficacy in long-term studies. (Fertil Steril® 2007;88:206–11. ©2007 by American Society for Reproductive Medicine.)

Key Words: Vessel sealing, bipolar coagulation, burst pressure, burst strength

Increasing numbers of minimally invasive laparoscopic procedures are being introduced into gynecological surgery (1, 2). Laparoscopic myectomy shortens hospitalization, accelerates recovery, lowers expenses, reduces pain, lessens blood loss, and decreases the extent of adhesions (3). In the laparoscopic management of ovarian remnants, electrocoagulation with bipolar forceps for ablation of tissue was less traumatic and decreased the number of recurrences, conversions to laparotomy, and postoperative complications (4). Nonetheless, an unacceptably high number of complications can occur with more complex laparoscopic interventions (5, 6).

Vessel sealing during laparoscopic procedures with electrosurgical methods using bipolar current has been widely introduced over the past decade (1, 7–10). Bipolar, sealing-induced hemostasis can withstand high intraluminal pressures (10–14) and therefore offers an alternative to suturing, resulting in reduced blood loss (15, 16). The seals are intrinsic to the vessel wall structure, are not adhesiogenic, and cannot be dislodged like some clips used for hemostasis (10, 17). The quality of vessel sealing, however, is often suboptimal (2, 9, 11). Manipulation to disengage the sealing instrument can weaken the seal (9). Excessive heat increases charring and stickiness of the sealed tissue, resulting in tissue necrosis and hemorrhagic complications (18). Thermal spread to

Received June 22, 2006; revised and accepted November 8, 2006.
Supported by the German Ministry of Education and Research (BMBF) (Berlin, Germany) within the project "Minimal invasive Technologie und Therapieverfahren" (grant 16SV 1352) and by a research grant from ERBE Elektromedizin GmbH (Tübingen, Germany).
Presented as a poster at the 33rd Annual Meeting of the American Association of Gynecological Laparoscopists in San Francisco, California, November 10–13, 2004. In addition, a summary of the findings was presented at the 14th Annual Congress of the International Society for Gynecological Endoscopy, London, United Kingdom, April 2–6, 2005.
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adjacent tissues can induce bowel injury, one of the most hazardous complications (2, 19, 20).

Conventional pulsed bipolar coagulation (CPC) has been shown to lead to adequate vessel sealing (3, 4, 8, 10, 11). The methods used so far, however, have been based on a pulse frequency not regulated by impedance but dependent on a preset relationship between pulse and pause duration. As tissue impedance increases because of thermal alteration, the current during the pulse decreases considerably and, after a certain point, even decreases to such an extent that the resulting heat energy, as dictated by Joule's Law, is too low to maintain the optimum tissue temperature needed for coagulation. During CPC, as tissue impedance continues to increase to higher levels, the fraction of the pulse during which current flow is not sufficient also lengthens.

We developed a new modulation of CPC in which, unlike CPC, an electrical feedback mechanism based on the degree of denaturation and desiccation of tissue regulates the duration of pulses and of pulse-pause sequences. As soon as current flow decreases to a defined level as a response to the increasing impedance, the pause and the next pulse are initiated automatically, thus avoiding longer fractions of pulses during which the current is too low to ensure the optimum tissue temperature. This dynamic time sequencing not only shortens the process as a whole, it also ensures a higher overall current. We termed this new modulation *intelligent, impedance-regulated, pulsed coagulation* (IPC). The aim of the present study was to establish whether the safety and reliability of vessel sealing with IPC is superior to that of CPC.

MATERIALS AND METHODS

The study was approved by the Research Programs Council of the University of Tübingen and the European Academy of the European Society of Gynecological Endoscopy. The authors advised ERBE Elektromedizin GmbH, Tübingen, Germany, on the development of the new technique, without any financial gain.

Study Design

One hundred thirty-two renal arteries were harvested from female Swabian Hall pigs (weight range, 45–55 kg). The specimens were dissected and the diameters determined. The vessels were thoroughly flushed with normal saline to remove all blood. They were then stretched across a titanium adapter and secured with purse-string sutures to a pressure application device. Figure 1 shows the experimental setup.

Three different modulations of electrothermal energy were applied to test their efficacy in vascular coagulation. The vessels were randomly assigned to a specific coagulation mode. Two were CPC modes with a tissue-independent pulse frequency: pulse duration of 800 ms, with a pause lasting 30 ms (CPC-I group; n = 50) or 300 ms (CPC-II group, n = 43). The third modulation (IPC group; n = 20) was designed to be intelligent and to regulate itself in a single coagulation procedure in response to the changing tissue impedance during thermal alteration (Fig. 2). In line with clinical practice, some additional vessels underwent multiple coagulation (three times) with CPC-I and CPC-II (MCPC-I and MCPC-II). The investigation therefore included five groups of vessels.

Vessel sealing was performed by using bipolar open forceps (ERBE BiClamp) and a pulsed high-frequency generator (ERBE VIO 300 D). Coagulation with the two-jawed clamp was triggered via a pedal, and visual and audio signals indicated that the process was completed. The pedal then was released. The system generator and the surgical instrument used in the experiment are already in extensive use in laparoscopic surgery. The forceps pressure was standardized by a sprung handle. During the heating process, all relevant information on current, voltage, and impedance was recorded digitally. The completed seal was visually inspected for discoloring, flatness, and translucency.

After sealing, the burst pressure was determined as a measure of seal quality by a different operator, who did not know which mode had been used to seal the vessel. Saline was infused to gradually increase the perfusion pressure by 20 mm Hg/s under constant pressure monitoring, until either the seal or the vessel wall burst. The pressure at which this occurred was defined as the burst pressure in millimeters of mercury. Vessels with seals that did not resist a pressure of 80 mm Hg were considered *instant seal failures,* and those that did not resist pressures of $\leq$200 mm Hg were considered *secondary seal failures*. Vessels with seals that resisted a pressure of 200 mm Hg were considered *successful seals.*

Statistical Analysis

Data are given as mean ± SEM. After demonstrating normal distribution and homogeneity of variance across groups, differences between groups (burst pressure) were calculated by one-way analysis of variance, followed by an appropriate post hoc test, including the correction of the alpha error to compensate for multiple comparisons. Fisher's exact test was used for the analysis of differences in rates and proportions (seal failures). Overall statistical significance was set at $P<.05$. The Statistical Package for Social Sciences (SPSS, version 11.5 for Windows; SPSS Inc., Chicago, IL) was used for statistical analysis.

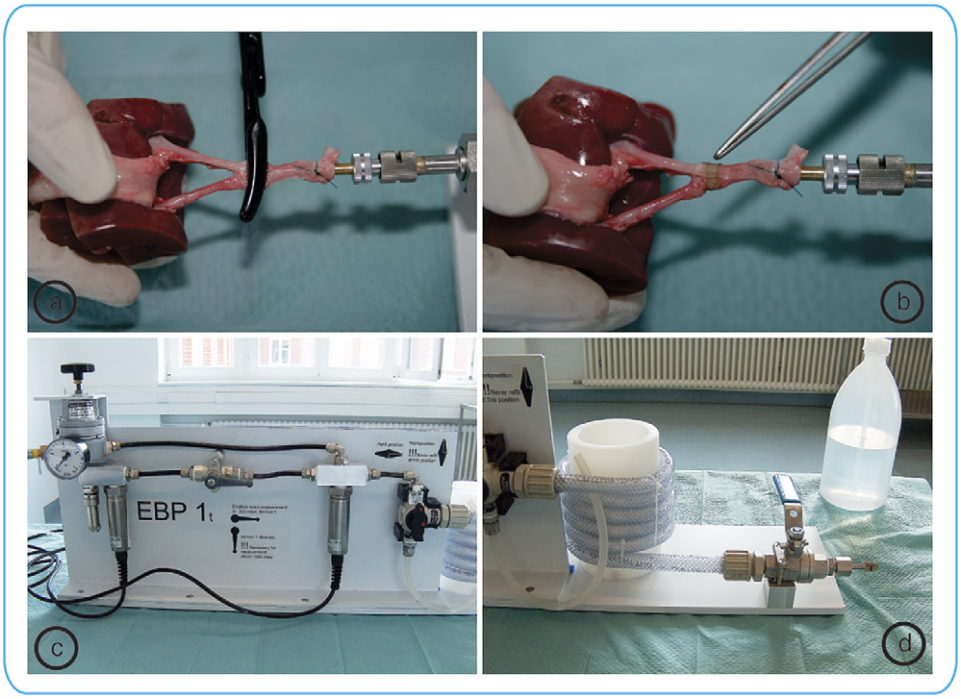
RESULTS

Application of Single CPC vs. Single IPC

In the first part of the study, single CPC-I (n = 50) and single CPC-II (n = 43) were compared with single IPC (n = 20). The mean vessel diameter did not differ between the three groups ($P>.05$; Table 1). The mean burst pressure achieved after IPC (585.5 ± 56.8 mm Hg) was significantly higher ($P<.001$) than that measured after CPC-I (372.6 ± 40.0 mm Hg) and CPC-II (334.2 ± 44.2). The pressures achieved

FIGURE 1

Coagulation process (**a** and **b**) and pressure application device (**c** and **d**).



Wallwiener. Intelligent pulsed vessel coagulation. Fertil Steril 2007.

in CPC-I–sealed arteries did not differ significantly from those observed in CPC-II–sealed arteries ($P>.05$).

In the IPC group, instant seal failures were seen in 5.0% of the vessels studied, and successful sealing was observed in 95.0%. In contrast, CPC-I produced 28.0% instant and 6.0% secondary seal failures, and only 66.0% of the single CPC-I seals were considered successful. Similar results were found for CPC-II, which produced 32.6% instant and 7.0% secondary seal failures, and only 60.5% of the single CPC-II seals were considered successful. Thus, the overall seal failure after IPC was significantly ($P<.05$) lower than that observed after CPC-I and CPC-II (Table 1).

Application of Multiple CPC vs. Single IPC

In the second part of the study, multiple CPC was compared with the results of single IPC (n = 20). Multiple CPC consisted of applying the coagulation mode three times in repetition. A total of 19 vessels were subjected to multiple coagulation, 9 of them with CPC-I and 10 of them with CPC-II. The mean vessel diameter did not differ between the three groups ($P>.05$; Table 2). The mean burst pressures achieved by these modes of coagulation did not differ significantly from that observed after single coagulation with IPC ($P>.05$; Table 2). In addition, both multiple CPC-I and multiple CPC-II did not produce any instant or secondary seal failures (Table 2). Accordingly, the rate of seal failures of multiple CPC was not different from that observed after single ICP.

Relationship Between Burst Strength and Vessel Diameter

A clear inverse relationship between burst pressure and vessel diameter was seen. The mean burst pressure of sealed blood vessels with diameters of <4 mm was 528.0 ± 58.6 mm Hg. Renal arteries with larger diameters (≥ 4 mm) showed a significantly ($P<.05$) lower burst pressure when compared with that measured in the smaller blood vessels (Fig. 3).

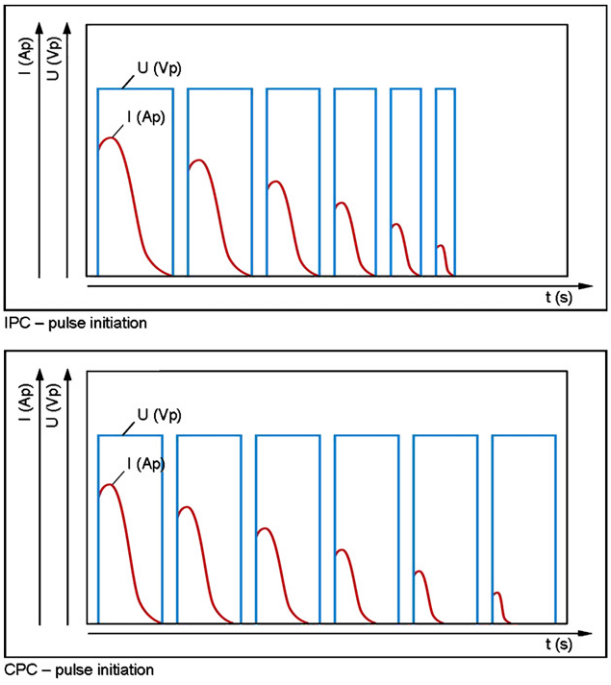
DISCUSSION

The widespread use of heating tissue to achieve coagulation is primarily driven by the availability of and improvement in technologies that perform this efficiently, but not by a detailed understanding of the bio-thermomechanics of the process (21). Improvements in instrumentation and technology have made a significant contribution to the consistent advances in laparoscopic surgery. Every improvement, however, generates new complications, and the introduction of energy-based vessel ligation has been no exception.

Thermal fusion is influenced by the amount of heat input over time and the length of time that the heat is applied. In bipolar coagulation, the interaction between impedance and current creates the high temperatures necessary for vessel

FIGURE 2

Pulse initiation in IPC (*upper panel*) and CPC (*lower panel*). In IPC, the pulses are initiated when current flow decreases to a certain level. Pulse initiation is therefore impedance dependent, whereas in CPC, pulse initiation is based on a predetermined relationship between pulse and pause.



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coagulation, and the change in tissue impedance indirectly indicates when this temperature has been reached. Pulsed coagulation appears to generate vapor zones with high impedance during the pulse. The current, seeking the path of lowest impedance, generates a high-energy density around these zones, leading to additional thermal effects. During the pause, while the forceps and the tissue are cooling, the vapor condenses and the moisture returns. During subsequent pulses, this process is repeated until uniform coagulation is achieved (7, 8). Denaturation of collagen is enhanced through hydration by decreasing its stability (21); therefore, continuous hydration during pulsed coagulation may very well lead to increased seal quality (7, 8).

Vessels can be successfully coagulated with CPC (8, 10, 11), but until now, most pulsed bipolar coagulation methods in current use are based on a predetermined pulse frequency with fixed bursts and pauses. They are dependent on impedance but do not regulate themselves on it. Because of the variability of vascular tissue and vessel size, this may result in overcoagulation or inadequate ligation, which both lead to seal failure.

Conventional coagulation can take $\leq$12 seconds and, in our study, resulted in seal failures in a high number of cases. We suggest that this is primarily a result of the long fraction of the pulse during which the current decreases and the energy density is reduced. This fraction increases as the coagulation process advances, whereas the pulse and pause intervals remain constant. The result is that the overall current and energy density is too low to ensure the high temperature and rapid increase in temperature that were required for thermal fusion.

To overcome this limitation, we developed a new modulation of the alternating current required, aiming to achieve IPC with a dynamic modulation process, in which the duration of pulses and pulse-pause sequences adapts itself to increasing tissue impedance. The adaptive initiation of subsequent pulses leads to a higher energy input per time unit and a more rapid rise in temperature, leaving the tissue to cool only during the pause designed for this very purpose. The present study evaluated the newly developed modulation of our coagulation software in an isolated porcine renal-artery model. We found that single application of our newly

TABLE 1

Coagulation of porcine renal arteries by using CPC, compared with IPC.

Parameter	CPC-I	CPC-II	ICP
Total no. of vessels (n)	50	43	20
Mean vessel diameter (mm)	4.52 ± 0.16	4.50 ± 0.17	4.20 ± 0.29
Instant seal failures, n (%)	14 (28.0)[a]	14 (32.6)[b]	1 (5.0)
Secondary seal failures, n (%)	3 (6.0)	3 (7.0)	0 (0.0)
Overall seal failures, n (%)	17 (34.0)[b]	17 (39.6)[b]	1 (5.0)
Successful seals, n (%)	33 (66.0)[b]	26 (60.5)[b]	19 (95.0)
Mean burst pressure (mm Hg)	372.6 ± 40.0	334.2 ± 44.2	585.5 ± 56.8

Note: Data are mean ± SEM.
[a] $P=.05$.
[b] $P<.05$ vs. ICP.

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TABLE 2

Coagulation of porcine renal arteries by using multiple CPC (mCPC), compared with by using IPC.

Coagulation mode	mCPC-I	mCPC-II	ICP
Total no. of vessels (n)	9	10	20
Mean vessel diameter (mm)	4.22 ± 0.39	4.25 ± 0.37	4.20 ± 0.29
Instant seal failures, n (%)	0 (0.0)	0 (0.0)	1 (5.0)
Secondary seal failures, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Successful seals, n (%)	9 (100.0)	10 (100.0)	19 (95.0)
Mean burst pressure (mm Hg)	597.3 ± 60.1	656.2 ± 56.5	585.5 ± 56.8

Note: Data are mean ± SEM and are not significantly different among the three groups.

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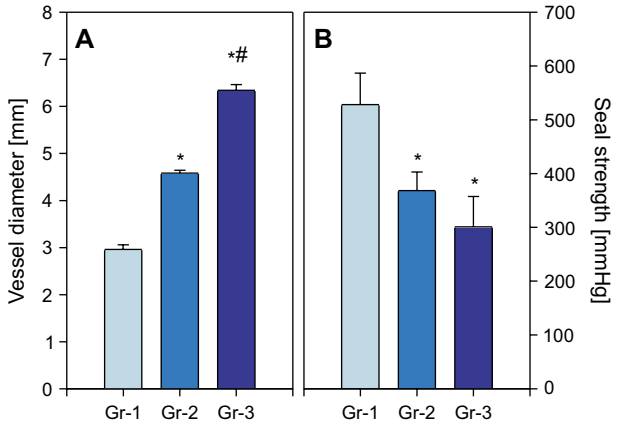
developed technique was significantly better than the coagulation methods in conventional use. Nonetheless, these results should be interpreted with caution until the results of in vivo follow-up studies are available.

One limitation of our study is that despite randomization, the operator was aware of the coagulation technique in use, thus introducing potential bias. However, most parameters that potentially could be influenced, such as the pressure applied and the duration of the sealing process, were standardized, and the different modes were used according to a randomized scheme. Only after preparing the vessel and before the sealing process did the operator change the mode according to the randomized study protocol. Another limitation of our study is the lack of follow-up data inherent in the model chosen. Also, the dissection of the arteries may have caused damage to the vessels tested. However, this appears unlikely, because all due care was taken.

FIGURE 3

Seal strength (**B**) given in relation to vessel diameter (**A**) after conventional or intelligent pulsed coagulation of porcine renal arteries. Data are grouped according to vessel diameter; that is, vessels with diameters <4 mm (group 1, *light blue bars*), ≥4 mm and <6 mm (group 2, *semiblue bars*), and ≥6 mm (group 3, *dark blue bars*). Note the inverse relationship, indicating decreasing seal strength with increasing vessel diameters. Data are mean ± SEM. *P<.05 vs. group 1. #P<.05 vs. group 2.



Wallwiener. Intelligent pulsed vessel coagulation. Fertil Steril 2007.

Unlike other study groups, we used bloodless coagulation in our experiments. To study the effect of the different current modulations on the vessel wall and the ensuing seal with only a minimum of variables, all blood was flushed from the vessel before testing. We believe that isolated preserved blood would not ideally mimic the clinical situation and that the specific effect of blood perfusion on the process of coagulation can be determined only in vivo.

Other reports have shown that arteries with a diameter of >5 mm show a higher rate of seal failures (11). In the present study, analysis of the relationship between vessel size and burst pressure confirmed that the seal quality was better the smaller the vessel, suggesting that the burst pressure decreases reciprocally with an increase in vessel diameter. Our results further show that IPC led to successful seals in 95% of vessels, whereas CPC-I led to only 66%, and CPC-II, only 61%. The number of seal failures in our study was higher than that in other studies, where only 5%–25% of seal failures occurred (8–10). This is probably because we used a very strict definition for failure (burst pressure of <200 mm Hg), because we believe that a rigorous approach is required when testing new technology. Moreover, in this study, vessels with relatively large diameters were studied, and all failures, including technical failures, were included in the analysis, which also would have contributed to the higher number of failures.

Multiple CPC in our experimental setting not only took longer but did not lead to a superior sealing quality than was achieved after single coagulation with IPC. It therefore appears that multiple coagulation is not superior to IPC, may cause more lateral thermal damage, and may carry a higher risk of rupture. However, because no prior reports on consecutive coagulation of vessels under experimental

conditions were found in the literature, and given the small number of vessels in this part of our study, the implications of these results should be viewed with caution.

In conclusion, this study in a porcine renal artery model demonstrates that in this specific setting, a new, intelligent, impedance-regulated modulation of energy-based vessel coagulation appears to achieve safer thermal fusion of vascular tissue than do commonly used methods. Unlike CPC, in which the coagulation process is preset by the operator, with IPC, the current flow is regulated by the impedance feedback from the tissue being coagulated. This promising technique requires further investigation in vivo, including long-term analyses.

Acknowledgments: The authors thank Ralf Klein, Dipl.-Ing., for technical support and Alistair Reeves, B.A., for editorial assistance.

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Instruments and Techniques

Thermal Conduction, Compression, and Electrical Current–An Evaluation of Major Parameters of Electrosurgical Vessel Sealing in a Porcine In Vitro Model

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ABSTRACT Bipolar vessel sealing is pivotal in laparoscopic hemostasis. However, major coaptive desiccation parameters have yet to be investigated in detail. The current investigation aims to study the impact of compressive pressure, thermal conduction, and electrical current effects on seal quality in a randomized, controlled experimental trial in an in vitro porcine model of vessel sealing. A total of 106 porcine vessels were sealed with either bipolar current or thermal conduction. Compressive pressure on the sealing site and maximum temperature were varied and monitored. Additionally, the longitudinal vessel tension was measured. The burst pressure of the resulting seal was determined as an indicator of seal quality. In bipolar coaptation, seal quality depends on the compressive pressure applied to the coagulation site in both arteries and veins. The optimal pressure interval was around 270mN/mm^2 for arteries and 200mN/mm^2 for veins. Deviation from these optimal pressures towards low and high extremes led to significantly fewer successful seals. We also found that both maximum coaptation temperature and vessel shrinking correlated with the seal quality. This correlation was reciprocal in arteries and veins. Thermal conduction alone was less successful than sealing by bipolar current. Therefore, compressive pressure during coaptation determines the seal quality. Upper and lower pressure boundaries for safe coaptation exist for both arteries and veins. Vessel sealing by thermal conduction without electrical current effects is possible but represents a less effective method for coaptation. These findings have implications for the rational design of new electrosurgical instruments. Journal of Minimally Invasive Gynecology (2008) 15, 605–610 © 2008 AAGL. All rights reserved.

Keywords: Bipolar coagulation; Thermal fusion; Vessel sealing; Compressive pressure

Laparoscopy was transformed from a diagnostic tool into a means for therapy by Kurt Semm, who performed the first laparoscopic appendectomy in 1983 [1]. Since then, laparoscopic operations have become routine. The clinical benefits of laparoscopy over laparotomy include shorter hospitalization, reduced pain, less blood loss, and decreased extent of

Dr. Enderle is medical director and head of research of Erbe Elektromedizin GmbH. Dipl.-Ing. Schäller is a scientist of the research department of Erbe Elektromedizin GmbH. Both provided important knowledge and information about the physics behind this topic and gave significant input to the study design. Analysis and interpretation of results were not influenced by them. All other authors, especially first and last author have no commercial interest. This investigation was supported by a grant from the German Ministry of Education and Research, Minimally Invasive Technologies and Techniques project (grant no. 16SV 1352) and by a grant from the Research Foundation of the Department of Obstetrics and Gynecology, University of Tuebingen, Tuebingen, Germany. This investigation was conducted at the laboratory facilities of the Department of Obstetrics and Gynecology, University of Tuebingen, Germany (Director: Prof. D. Wallwiener) and the Research Facility of ERBE Electromedicine, Tuebingen, Germany.
Presented at the 15th Annual Congress of the European Society of Gynecological Endoscopy in Strasbourg, France, October 5–7, 2006.
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Submitted January 25, 2008. Accepted for publication May 3, 2008.
Available at www.sciencedirect.com and www.jmig.org

1553-4650/$ - see front matter © 2008 AAGL. All rights reserved.
doi:10.1016/j.jmig.2008.05.003

adhesions [2]. This success has depended on continuous improvements in technology, with the introduction of electrosurgical vessel sealing probably being the most fundamental [3–5]. For many applications it is more efficient than alternative methods for ligation (e.g., suture, hemoclips, ultrasonic coagulation shears) [6,7].

Specific biothermomechanical parameters are key to the success of electrosurgical vessel sealing. In particular, the applied temperature and compressive pressure (CP) are thought to be pivotal factors [8,9]. In addition, tissue shrinking occurs during the sealing process. Very little detailed analyses of these parameters are available today. Moreover, it is not clear whether or not the applied high-frequency electrical current acts independently from the resulting increase in temperature.

The current study evaluates the influence of CP and temperature on electrosurgical vessel sealing in an in vitro setup. The relationship between achieving a good quality seal and mechanical vessel contraction was investigated. Finally, the effects of heat generated by purely thermal conduction as opposed to the high-frequency effects induced by electrical current are shown.

Methods

The study protocol was approved by our university research programs council and the European Academy of the European Society of Gynecological Endoscopy.

An experimental setup (Fig. 2) was designed that allowed standardized coaptation using either bipolar electrical current or purely thermal conduction without electrical current. The vertical CP during coaptation could be altered independently. All experiments were performed in an incubator at 36°C and 90% humidity to mimic physiologic conditions. For the sealing process, 2 pairs of jaws with rounded edges, simple surface geometry, and low thermal capacity were purposely built to include temperature probes (Nickel-Chrome-Elements Type K; Reckmann Measurement Technology, Hagen, Germany) 0.1 mm beneath the surface. One pair of jaws, made from polished stainless steel, was connected to a bipolar generator (Vio 300D; ERBE Electromedicine, Tuebingen, Germany). The generator was used with the clinically established pulsed biclamp mode (automatically modulated sine-wave signal form with a fundamental frequency of 350 kHz as previously described [10] and comparable with Valleylab's LigaSure [7] [Boulder, CO] and Gyrus' PlasmaKinetic [11] pulsed bipolar systems [Maple Grove, MN]) and autostop. The other pair of jaws was made from a silver-silicon compound (Wielandin GmbH, Pforzheim, Germany) to include a platinum microheater (Heraeus Sensor-Nite GmbH, Kleinostheim, Germany) for vessel sealing by purely thermal conduction. Here the coaptation process was stopped after all visual and acoustic signs of vaporization had ceased. Both pairs of jaws were built into a pressure device capable of impinging the vessel with a defined vertical CP. The jaws had a width of 6 mm, which defined the area of coagulation. The CP was applied through a metal bellow (Hydra-Metallbalg; Witzenmann GmbH, Pforzheim, Germany) filled with precision-controlled compressed air. A resistance strain gauge (ME-Messsystem GmbH, Henningsdorf, Germany) served as a force sensor to detect changes as small as 0.01 N.

A total of 106 porcine vessels (58 renal, femoral, and carotid arteries with a mean caliber of 5.1 mm [SD 1.3, CI 0.3] and 48 veins with a mean caliber of 4.8 mm [SD 1.5, CI 0.4) were harvested by the same investigator from 5 female Swabian-Hall pigs. The harvested vessels were randomized for the experiments. In all, 59 vessels were used to investigate the correlation between CP during bipolar electrocoaptation and seal quality (Table 1). The remaining 47 vessels were used to study vessel sealing by purely thermal conduction (Table 1). All vessels were carefully dissected from their connective tissues in situ and thoroughly flushed with a solution designed to protect transplants during transport, to remove any residual blood. The vessels then remained in a solution bath.

Immediately before sealing, the respective vessel was warmed in a 36°C saline bath and attached to the experimental apparatus by connectors (Luer-Lock; Volzer

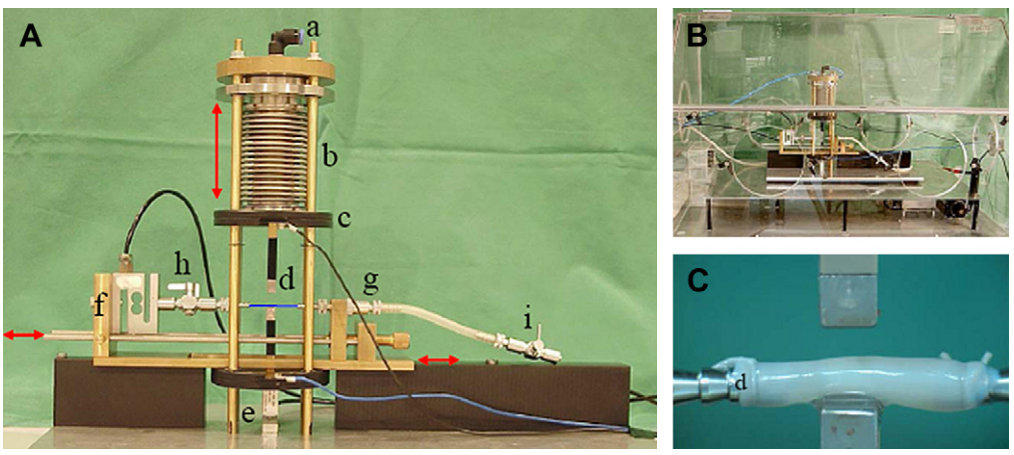

Fig. 1. Experimental setup with application of CP (A) and in incubator (B). Vessel in coaptation device (C).

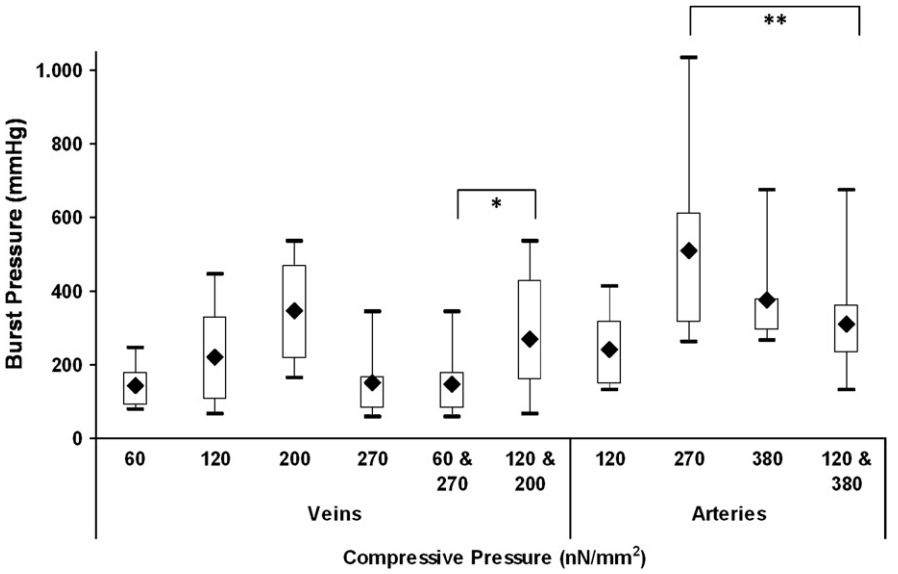

Fig. 2. Burst pressure (BP) in bipolar vessel sealing with varying CP. Mean BP in mm Hg is shown for each group. Means (*diamonds*), interquartile ranges (*boxes*), and maximum and minimum values (*error bars*) are indicated. *p = .017, **p = .022.

Medizintechnik, Tuttlingen, Germany). The vessel was then inflated with saline to a constant internal pressure of 100 mm Hg for arteries and 30 mm Hg for veins to unfold the collagen fibers and the endothelium. Subsequently, the vessels were assigned a number according to the order of harvesting and randomized in blocks to the experimental groups so that each group contained vessels from all of the pigs. Table 1 depicts the study groups.

Burst pressure (BP) was determined as a measure of seal strength immediately after coaptation. Saline was infused gradually to increase the perfusion pressure by 20 mm Hg/s until the seal burst. Vessels that did not resist a pressure greater 100 mm Hg for arteries or 30 mm Hg for veins were considered instant seal failures. Those vessels that did not resist pressures of 250 mm Hg for arteries or 80 mm Hg for veins were considered secondary seal failures. Sustaining a pressure greater than 250 mm Hg and 80 mm Hg, respectively, was the definition of a successful seal. For calculation of the mean BP, both successful seals and secondary failures were taken into account. In addition, CP, temperature, and changes in longitudinal vessel tension were digitally recorded during the duration of the experiment with a measuring board (ME-2600i PCI; Meilhaus Electronic, Puchheim, Germany) and software (Labview 7.0; National Instruments, Austin, TX).

Statistical Analysis

Differences between groups were analyzed nonparametrically by the Wilcoxon test or Kruskal-Wallis test for continuous variables. Other variables were tested by the χ^2 test. Correlations between variables were investigated

Table 1
Study groups and parameters

Experiment	Vessels	Group	Compressive pressure (mN/mm^2)	No. of vessels	Caliber	Temperature
Compressive pressure in bipolar electrocoaptation	Arteries	I-A1	120	11	4.7	Automatic
		I-A2	270	11	5.1	Automatic
		I-A3	380	10	5.2	Automatic
	Veins	I-V1	60	7	4.6	Automatic
		I-V2	120	6	4.2	Automatic
		I-V3	200	7	4.4	Automatic
		I-V4	270	7	4.5	Automatic
Coaptation through thermal conduction	Arteries	II-Arandom	300–800	10	5.1	140–220
		II-A1	500	4	5.5	125
		II-A2	500	4	5.3	155
		II-A3	500	4	5.5	185
		II-A4	600	4	5.1	155
	Veins	II-Vrandom	40–600	5	5.1	125–205
		II-V1	150	6	5.5	155
		II-V2	250	5	5.0	155
		II-V3	300	5	5.6	155

nonparametrically after Spearman. The significance level was set to .05. For post hoc tests the significance level was adjusted according to Bonferroni. Results are presented as means ± the corresponding 95% confidence interval. The statistical analysis was done with software (JMP, Version 5.1.2; SAS Institute Inc., Cary, NC).

Results

No statistically significant differences existed in vessel caliber between the test groups. Neither did the anatomic origin of the vessels have any significant influence on the experimental outcomes or correlation with another study parameter nor did an individual animal.

Seal Failures after Bipolar Electrocoaptation with Different CP

In arteries, the incidence of all seal failures (both initial and secondary) after bipolar coaptation was 72.7% for a CP of 120 mN/mm^2 (I-A1), decreasing to 0% for a CP of 270 mN/mm^2 (group I-A2), and then increasing again to 20.0% for 380 mN/mm^2 (I-A3). These differences were statistically significant ($p = .002$). In veins the differences in seal failures for different CPs were not statistically significant for the number of samples studied ($p > .05$) (Table 2).

BP after Bipolar Electrocoaptation with Different CP

In arteries, the mean BP was 243 mm Hg (SD 112, CI 83) for CP of 120 mN/mm^2 (I-A1), increasing to 510 (SD 233, CI 138) with CP of 270 mN/mm^2 (I-A2), and then decreasing back to 375 mm Hg with CP of 380 mN/mm^2 (I-A3). These differences were statistically significant ($p = .022$) (Fig. 2). In the venous samples, the differences between the individual groups were not statistically significant for the number of samples studied. For both arteries and veins, the combined BP for the intermediate groups were compared with the combined BP for the extreme groups. For veins, the BP of the extreme groups (I-V1 [60 mN/mm^2] and I-V4 [270 mN/mm^2]) was 147 mm Hg (SD 87, CI 51), which contrasted significantly with 290 mm Hg (SD 153, CI 95) for the intermediate groups (I-V2 [120 mN/mm^2] and I-V3 [200 mN/mm^2]) ($p = .017$). For arteries, the BP for the intermediate group I-A2 (270 mN/mm^2), 510 mm Hg (SD 233, CI 138), was significantly higher than 309 mm Hg (SD 139, CI 73) for the extreme groups I-A1 (120 mN/mm^2) and I-A3 (380 mN/mm^2) ($p = .022$).

Analysis of the maximum temperature (mT) values for seal failures and successes from all groups with bipolar vessel sealing revealed significant differences. In arteries, the mean mT of all successful bipolar seals, 119°C (SD 10, CI 4), was significantly higher than the mean mT of all seal failures, 105°C (SD 17, CI 10) ($p = .011$). In contrast, the mean mT for successful bipolar seals in veins, 116°C (SD 13, CI 6), was significantly lower than the mean mT for seal failures, 127°C (SD 10, CI 7; $p = .038$) (Fig. 3). Also, in both arteries and veins, mT correlated positively with CP (correlation coefficient after Spearman: rho = 0.83 and $p < .001$ in arteries and rho = 0.54 and $p = .004$ in veins) (Table 2).

The changes in longitudinal tension exerted by the vessels for arteries and veins were calculated for successful seals and for seal failures after bipolar electrocoaptation and coaptation by purely thermal conduction. The mean difference in longitudinal tension before and after coaptation (ΔlT) for successful arterial seals was 1.4 N (SD 1.3, CI 0.5). In contrast, the tension change in seal failures was only 0.8 N (SD 0.6, CI 0.2). These differences were significant ($p = .019$) (Fig. 3). For veins, the mean difference in ΔlT between successful seals and failures was not statistically significant ($p > .05$) (Table 2).

Vessel Sealing with Coaptation by Purely Thermal Conduction

Of 26 arteries sealed purely by thermal conduction without electrosurgical effects, only 5 (19.2%) successful seals occurred. Sealing of veins by purely thermal conduction resulted in 11 successful seals of 21 (52.4%). Thus, vessel sealing by thermal conduction was less successful than bipolar electrocoaptation for equivalent temperatures. No statistically significant difference occurred in the mean temperature of the successful seals (167°C [SD 32, CI 28] in arteries and 162°C in veins [SD 18, CI 11]) compared with the mean temperature of seal failures (164°C in arteries [SD 28, CI 12] and 154°C in veins [SD 12, CI 8]) ($p > .05$).

Discussion

The current study investigates the influence of CP, temperature, tissue shrinkage, and electrical current versus

Table 2
Compressive pressure in bipolar coaptation

	Group	Compressive pressure (mN/mm^2)	No. of vessels	Successful seals	Initial failures	Secondary failures	Maximum temperature (°C)	Delta in vessel tension start-end (N)
Arteries	I-A1	120	11	3	4	4	100 (SD 11, CI 7)	0.7 (SD 0.7, CI 0.4)
	I-A2	270	11	11	0	0	119 (SD 8, CI 5)	1.8 (SD 1.7, CI 1.1)
	I-A3	380	10	8	2	0	126 (SD 6, CI 4)	1.4 (SD 1.0, CI 0.7)
Veins	I-V1	60	7	4	1	2	110 (SD 7, CI 6)	0.7 (SD 0.7, CI 0.6)
	I-V2	120	6	4	0	2	115 (SD 10, CI 7)	0.9 (SD 1.2, CI 1.1)
	I-V3	200	7	3	3	1	127 (SD 13, CI 10)	2.6 (SD 1.5, CI 1.1)
	I-V4	270	7	4	2	1	127 (SD 16, CI 11)	2.4 (SD 1.1, CI 0.8)

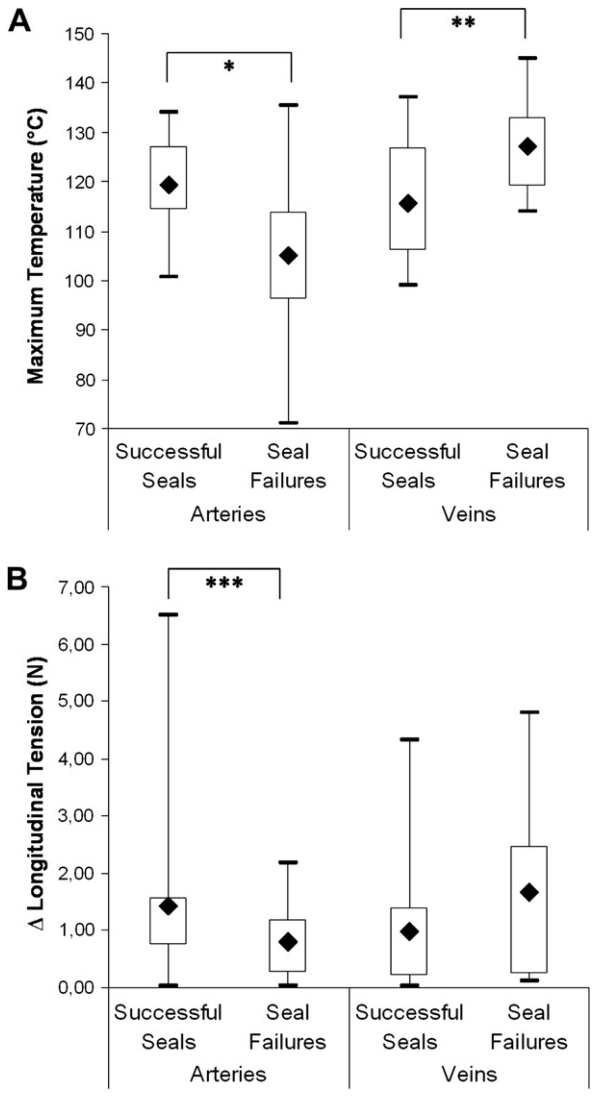

Fig. 3. Correlation between sealing success and mT respectively changes in longitudinal vessel tension (ΔLT). (A) Mean MT is shown in °C for all successful seals and all seal failures broken down by arteries and veins. (B) Mean ΔLT is shown in N analog to *A*. Means (*diamonds*), interquartile ranges (*boxes*), and maximum and minimum values (*error bars*) are indicated. *p = .011, **p = .038, ***p = .019.

thermal conduction on successful vascular sealing. At this, the calibers of the sealed porcine vessels (arteries 5.1 mm and veins 4.8 mm) were comparable with the human uterine artery, which ranges from 3 to 5 mm in diameter [12].

The amount of CP during the coaptation process significantly influenced the quality of the achieved seal. In arteries especially, too low a CP led to a high number of seal failures. Increasing CP resulted in a greater number of successful seals and a higher mean BP. This shows that a minimum threshold CP exists, below which safe coaptation cannot be guaranteed. On the other hand, increasing CP excessively also reduced the seal quality. This shows an upper limitation to the CP, above which the vessel wall is damaged in such a way that optimal coaptation can no longer be achieved. Consequently, a limited CP interval exists for safe vessel sealing. For arteries, this interval is around 270 mN/mm^2. In contrast, the optimal pressure for veins is around 200 mN/mm^2. This may reflect their lower wall thickness (Fig. 2).

In successful arterial seals, the mT during coaptation was significantly higher than in seal failures (Fig. 3A). This indicates a positive correlation between temperature and seal stability. In veins, however, the situation was reversed with the mT for successful seals significantly lower than for seal failures. These findings indicate that, similar to the situation for CP, a specific optimal temperature for bipolar coaptation exists. The optimal temperature appears to depend on the thermal capacity of the vessel, as determined by its wall thickness, and this may account for the differences between arteries and veins.

Another important consideration is that vessels shrink in response to thermal fusion and water vaporization. The difference in longitudinal arterial tension before and after coaptation (ΔlT) differed significantly between successful seals versus seal failures (Fig. 3B). This indicates that vessel contraction correlates with the strength of the seal and, therefore, represents an indirect indicator of coaptation success.

In principle, it is possible to seal both arteries and veins through purely thermal conduction without application of an electrical current. The effectiveness of this method was only 19.2% for arteries and 52.4% for veins, which is considerably less than what was achieved by bipolar electrocoaptation.

The amount and rate of heat deposition, coupled with the properties of the sealed vessel, govern the resulting changes at the microscopic and macroscopic scale [13]. Coaptation by purely thermal conduction dries the vessel wall from the outside to the inside and consequently results in a dry outer layer around the still moist inner layers with a differential heat distribution over the cross section of the vessel wall. This differential in cross-sectional heat distribution would increase with thermal capacity of the vessel wall and temperature. As a result, it is particularly great for the thick-walled arteries leading to inadequate results. In contrast, bipolar electrocoaptation heats the vessel layers simultaneously through their impedance to the electrical current rather than by thermal conduction from the outside. The instant dissipation of energy into the tissue by electrocoaptation compares favorably with the relatively slow dissipation of energy by thermal conduction. In light of our presented data and these theoretic considerations, it is not efficient to use coaptation by thermal conduction in lieu of electrocoagulation in a clinical setting.

The current study has 3 limitations that represent directions for future study. First, the results should be validated in the presence of blood in vivo. Second, the number of vessels was rather small in this pilot study and should be increased in further studies. Third, the integral of the temperature-time curve should be determined in addition to mT. This will shed light on time as a factor in electrosurgical vessel sealing. Moreover, the presented results necessitate further analysis of CP and temperature in instruments that are already in clinical use. Data on these matters are scarce but necessary as the basis for future instrument designs.

In summary, the current investigation defines the optimal CP interval during vessel sealing for arteries and veins. An association among temperature, bipolar high-frequency effects, and successful sealing is shown. These findings have implications for the rational design of future electrosurgical instruments.

The authors wish to thank Dipl.-Ing. Sven Egner and Dipl.-Ing. Christian Seier for technical assistance and Drs. Ulf Ellwanger and Holger Lüdtke for support with the statistical analysis.

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The extent of adhesion induction through electrocoagulation and suturing in an experimental rat study

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Objective: To investigate the effect of three types of peritoneal trauma occurring during surgery (high-frequency bipolar current, suturing, and mechanical damage) on postoperative adhesion formation in a rodent animal model.
Design: Randomized, controlled experimental trial in an in vitro animal model.
Setting: Laboratory facilities of a university department of obstetrics and gynecology.
Animal(s): Thirty-five female Wistar rats.
Intervention(s): Bilateral experimental lesions were created on the abdominal wall in every animal. The effect of minimal electrocoagulation was examined by creating lesions (n = 14) through sweeps of a bipolar forceps with a duration of 1 second and standardized pressure. For extensive electrocoagulation standardized lesions (n = 14) were created using sweeps of a duration of 3 seconds and three times more pressure. For mechanical trauma, standardized lesions (n = 14) were created by denuding the peritoneum mechanically. To study the additive effect of suturing, experimental lesions were created by suturing plus minimal electrocoagulation (n = 14) or mechanical denuding (n = 14).
Main Outcome Measure(s): Adhesion incidence, quantity, and quality of the resulting adhesions were scored 14 days postoperatively. Adhesions were studied histopathologically.
Result(s): Mechanical denuding of the peritoneum did not result in adhesion formation. After minimal electrocoagulation, mean adhesion quantity of the traumatized area averaged 0%. This contrasted with extensive electrocoagulation, where there was 50% adhesion. Additional suturing increased mean adhesion quantity to 73% and 64% for superficial electrocoagulation and mechanical denuding, respectively.
Conclusion(s): We conclude that superficial trauma limited mostly to the parietal peritoneum may be a negligible factor in adhesion formation in this model. This appears to be irrespective of the mode of trauma. However, additional trauma to the underlying tissues, either by deeper electrocoagulation or suturing, leads to significantly increased adhesion formation. These data also show that there is a spectrum of electrocoagulation trauma at the lower end of which there is little adhesion formation. (Fertil Steril® 2009;■:■–■. ©2009 by American Society for Reproductive Medicine.)

Key Words: Adhesions, bipolar coagulation, adhesion induction, suturing

Adhesions occur after abdominal and pelvic surgery in more than 70% of cases (1). Patients who have developed postoperative adhesions are at risk of serious complications, including intestinal obstruction (2) and infertility (3). If adhesiolysis is required, the affected patients are additionally exposed to the risks and complications of re-surgery and anesthesia. Adhesions also place a burden on surgeons due to prolonged subsequent operations (4), which are potentially associated with greater risk of enterotomy (5). Finally, there is a considerable financial burden on the health system with the cumulative costs over 10 years of adhesion-related readmissions in the United Kingdom estimated at £569 million (6).

It is generally believed that adhesion formation occur secondary to peritoneal damage with a subsequent imbalance in peritoneal fibrinolysis (7, 8). Suturing was shown to induce adhesion formation and it has been hypothesized that this is secondary to ischemia (9). Similarly, electrocoagulation is known to predispose to adhesion formation (7, 10). Yet electrocoagulation is used extensively to achieve hemostasis. Therefore, a thorough analysis of the relationship between electrocoagulation and adhesion formation is indicated.

Received July 22, 2008; revised November 23, 2008; accepted December 2, 2008.
C.W.W. has nothing to disclose. B.K. has nothing to disclose. M.W. has nothing to disclose. C.B. has nothing to disclose. K.B.I. has nothing to disclose. T.K.R. has nothing to disclose.
Supported a grant from the German Ministry of Education and Research, Minimally Invasive Technologies and Techniques project (grant no. 16SV 1352) and by a grant from the Research Foundation of the Department of Obstetrics and Gynaecology, University of Tübingen, Tübingen, Germany.
Presented at the 15th Annual Congress of the European Society of Gynecological Endoscopy (ESGE) in Strasbourg, France, October 5–7, 2006, and at the 36th Annual Meeting of the American Association of Gynecological Laparoscopists in Washington, D.C., November 14–17, 2007, as an oral presentation.
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In the current study we investigate the hypothesis that the extent of trauma with electrocoagulation results in varying degrees of postsurgical adhesion and study the additive effect of suturing.

MATERIALS AND METHODS

Institutional Review Board (IRB) approval was obtained. A total of 35 female Wistar rats (Charles River Laboratories, Sulzfeld, Germany) with a weight range of 220–280 g were housed under standardized laboratory conditions that were in keeping with the European requirements. All operations to induce adhesions were performed by the same surgeon under aseptic conditions. Anesthesia was induced by nebulized isoflurane, and intraperitoneal (IP) ketamine (100 mg/kg) and xylazin (5 mg/kg). The concentration of the injected ketamine was 100 mg/mL, and the concentration of the injected xylazin was 20 mg/mL. The peritoneal cavity was opened by a 4-cm midline incision. Subsequently, the animal was allocated to one of the five experimental groups (Table 1) according to a permutated block randomization plan. Both lateral body walls of the animal were then traumatized accordingly. Per session, one animal from each group was operated.

Adhesion Induction

All traumatization was inflicted by the same surgeon. Standardization of the traumatized area was achieved using rectangular plastic stencils with cut-out centers of the sizes of the intended trauma. The applied pressure was standardized using electronic scales, which were placed underneath the tissue being traumatized (Fig. 1).

In group 1, minimal electrocoagulation, standardized lesions were inflicted on an area of 2.5 × 2 cm by sweeping bipolar forceps over the abdominal peritoneum. The forceps were fixed open so that both branches and open distances measured exactly 0.5 cm and each sweeping was done exactly below the previous one. The time for each sweeping was 1 second and the pressure that was applied on the tissue by the forceps during each sweeping amounted to 15 × *g*. The generator was set to 60 W. For all electrocoagulation, bipolar coagulation forceps (Coagulationforceps "normal length"; ERBE Elektromedizin, Tübingen, Germany) and a Vio 300D bipolar generator (ERBE Elektromedizin) were used, which are among the standard instruments in our hospital. In group 2, extensive electrocoagulation, traumatization was achieved similarly to group 1 but each sweeping lasted 3 seconds and the pressure on the tissue amounted to 45 × *g*. In group 3, narrow stripes of 2 × 0.5 cm area were created as for group 1 but with additional suturing through the underlying musculature, approximately 1 mm deep, with five interrupted sutures (3/0 polyglactin; Ethicon, Somerville, NJ) placed equidistantly over the peritoneal defect (11, 12). The tension of the sutures was chosen to simulate approximation of the peritoneum during wound closure. In group 4, the peritoneum was carefully incised and stripped off the musculature over an area 2.5 × 2 cm. In group 5 narrow stripes of 2 × 0.5 cm were created as for group 4 but with additional suturing as in group 3. These models were chosen to replicate the different aspects of peritoneal trauma during surgery (electrocautery, suturing, sharp incision, and mechanical damage). The relatively large area of 2.5 × 2 cm chosen to examine adhesion formation after minimal electrocoagulation or extensive electrocoagulation was decided upon because previous pilot experiments of denuding or minimal electrocoagulation resulted in little adhesion formation. To combine the modalities electrocoagulation and suturing and thereby mimicking the situation in the human operating theater, smaller areas were coagulated, thus enabling the surgeon to overstitch the lesion. The smaller traumatized area was of no concern for the investigators, as previous pilot experiments led them to believe that suturing would greatly increase adhesion formation (11, 12).

Complete hemostasis was achieved using pressure from a sterile swab. Subsequently the midline incision was closed in two layers with continuous 3/0 polyglactin. The duration of each surgery was approximately 20 minutes from incision to closure of the skin. Postoperatively the animals received 0.05 mL of buprenorphine (0.05–0.1 mg/kg) SC as soon as the animals' whiskers started moving after the operation

TABLE 1

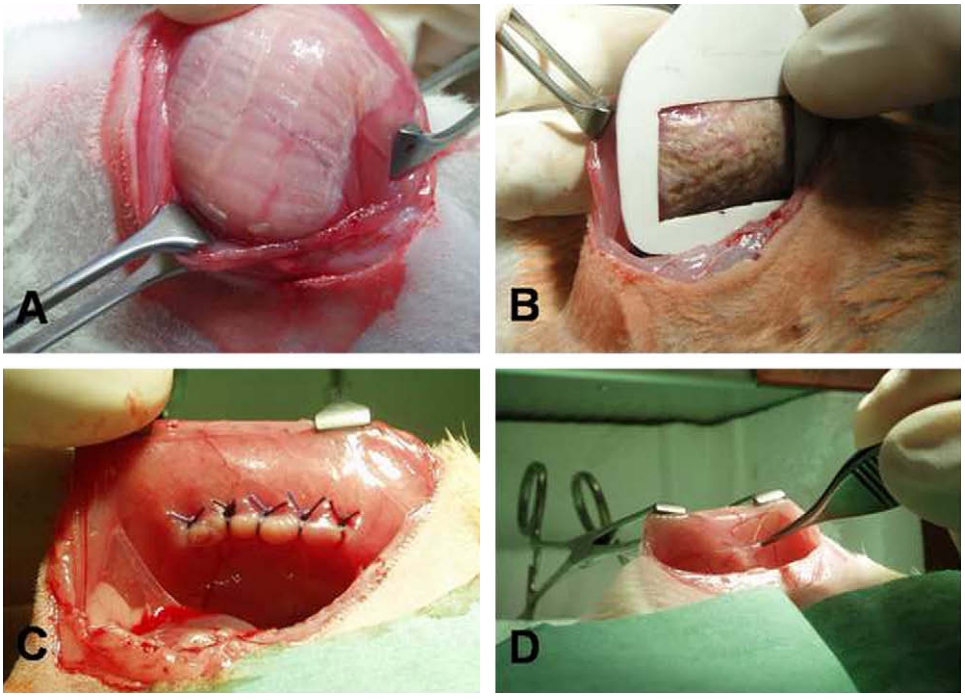
Study groups.

Group	Trauma	Number of traumatized areas	Number of animals
1	Minimal electrocoagulation	14	7
2	Extensive electrocoagulation	14	7
3	Minimal electrocoagulation + suturing of the underlying musculature	14	7
4	Trauma of the peritoneum only by mechanical denuding of the peritoneum	14	7
5	Trauma of the peritoneum only by mechanical denuding + suturing of the underlying musculature	14	7

Wallwiener. Electrocoagulation and adhesion formation. Fertil Steril 2009.

FIGURE 1

Adhesion induction. Minimal coagulation (**A**), extensive coagulation (**B**), minimal coagulation plus suturing (**C**), and mechanical peritoneal denuding (**D**).



Wallwiener. Electrocoagulation and adhesion formation. Fertil Steril 2009.

and then four times per day for 3 days. Afterward the animals were observed daily for signs of complications. After 14 days the animals were sacrificed using CO_2.

Adhesion Scoring

The adhesion scoring was performed immediately after euthanasia by a pathologist to whom the allocation of each animal was blinded. Adhesion incidence in percent was defined as the number of trauma sites at which adhesions developed postsurgically. Adhesion quantity was defined as the adhesion-covered area divided by the area of the traumatized area. Adhesion quality was considered "filmy" if the scale of a ruler was visible through the tissue, otherwise it was considered "dense" (11).

Histopathology

All traumatized areas were excised en-bloc together with any adhesive tissue and fixed in 4% phosphate-buffered saline (PBS)-buffered formalin. After routine tissue processing, histologic evaluation was done by hematoxylin and eosin (H & E) staining, Elastica van Gieson and Goldner staining for fibrous tissues, as well as Pears staining for fibrin.

Statistics

Adhesion incidence was analyzed using Fisher's exact test. Statistical significance in adhesion quantity and quality was tested using Kruskal-Wallis analysis with Bonferroni correction to protect the overall error rate against multiple significance tests. The significance level was set to $\alpha = 0.05$. The statistical analysis was done with a statistics package (JMP, Version 5.2.1; SAS Institute Inc., Cary, NC) and *R* (R Development Core Team, Vienna, Austria).

RESULTS

Adhesion Incidence

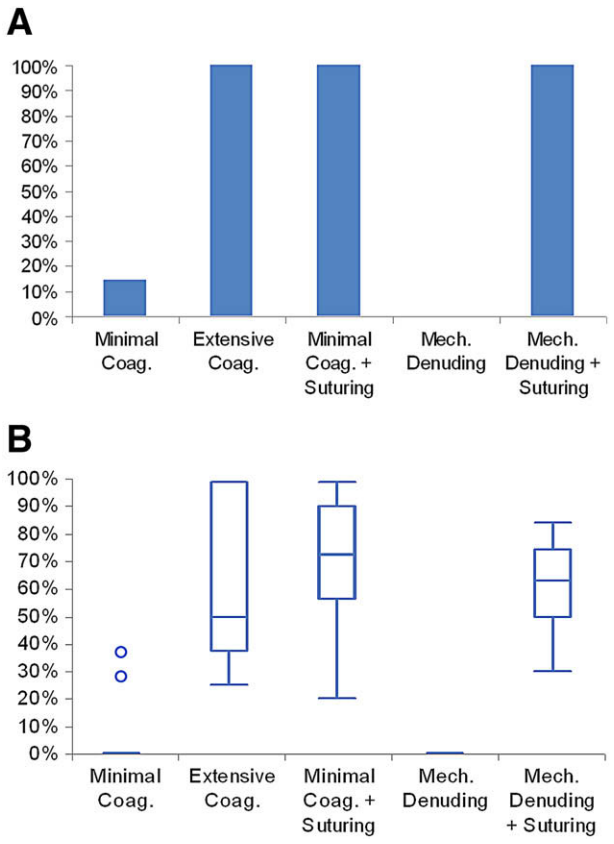
Adhesions developed in 14% (n = 2) of areas traumatized by minimal electrocoagulation. There were adhesions at all traumatized sites after extensive electrocoagulation (n = 14) and in the two groups involving suturing (n = 14, n = 14). There were no adhesions in the mechanical denuding group. Fisher's exact test revealed that these differences were highly significant ($P<.001$) (Fig. 2).

Adhesion Quantity

Minimal electrocoagulation caused a median adhesion quantity of 0% (range 0–37%). Extensive electrocoagulation led to 50% adhesion quantity (range 25%–100%). This difference was highly significant ($P<.001$). Minimal electrocoagulation with additional suturing of the underlying tissue resulted in 73% adhesion quantity (range 20%–100%). Here the difference against minimal electrocoagulation

FIGURE 2

Adhesion scores. Incidence of postsurgical adhesion formation (**A**, bars represent the adhesion formation in percent of trauma for each group) and the adhesion quantity (**B**, the boxplots indicate minimum observation, first quartile, median, third quartile, and maximum observation. According to standard convention observations 1.5 times the interquartile range lower than the first quartile or 1.5 times the interquartile range higher than the third quartile are considered outliers and indicated by circles).



Wallwiener. Electrocoagulation and adhesion formation. Fertil Steril 2009.

alone was also highly significant ($P<.001$). Peritoneal denuding caused no adhesions (range 0–0). If the underlying musculature was also sutured there was 64% adhesion quantity (range 30%–85%). This difference was also highly significant ($P<.001$). There was no statistically significant difference between denuding and minimal electrocoagulation (Fig. 2).

Adhesion Quality

The omental and pelvic fat were the only tissues attached to the traumatized areas, with the only exception of two cases when intestine was included after extensive coagulation. Electrocoagulation trauma only, both minimal and extensive, resulted in only dense adhesions. After minimal electrocoagulation plus suturing dense adhesions covered 26% (range 0–83%) of the traumatized areas, whereas filmy adhesions covered 39% (range 0–94%). After peritoneal denuding plus suturing dense adhesions covered 35% (range 10%–75%) of the traumatized areas, whereas filmy adhesions covered 15% (range 0–55%). Kruskal-Wallis one-way analysis of variance demonstrated significant differences between the groups ($P<.001$).

Histopathology

In the case of extensive electrocoagulation histology revealed damage not only to the serosal membrane but to the subserosa and the underlying musculature affected. In addition, edema in the subserosal connective tissue, hyperemia of the small vessels, and leukocytic infiltrate of the subserosa including the underlying musculature with destruction of muscle cells and beginning fibrous organization could be demonstrated. These effects were rarely seen with minimal electrocoagulation, but in all cases of extensive coagulation. Animals with mechanical peritoneal denuding showed no changes in the underlying musculature. Animals with suturing had granuloma formation and foreign body reaction in the musculature.

DISCUSSION

The current study investigates the extent of adhesion induction through electrocoagulation, suturing, and mechanical trauma of the peritoneum and the abdominal wall in an experimental rodent model.

In our study, mechanical denuding of the peritoneum without damage to the underlying musculature caused no adhesion formation. After minimal electrocoagulation, adhesion incidence of the traumatized area was only 14%, whereas after extensive electrocoagulation, minimal electrocoagulation with suturing or mechanical denuding with suturing the adhesion incidence was 100%. This is despite the narrower traumatized areas in the sutured groups, which was chosen to allow suturing over the entire defect (11).

As for adhesion quantity, there was minimal quantity with minimal electrocoagulation but ample quantity for all other modalities except for denuding without surgery, which resulted in no adhesion formation.

In this study, trauma by electrocoagulation resulted only in dense adhesions, whereas when either coagulation or denuding was combined with suturing of the underlying musculature both filmy and dense adhesions were found. Further investigation is needed to determine whether the mode or the extent of traumatization correlates with adhesion quality, especially because the clinical relevance of adhesion quality (filmy vs. dense) is not yet clear.

Electrocoagulation significantly differs from other modalities used in experimental models with regard to the quality of the injury produced. On the one hand, the thermal spread

leads to damage to deeper structures. On the other hand, electrocoagulation leads to sealing of blood vessels, which is not a feature of mechanical injury by abrasion with a brush or excision of the peritoneum. In spite of this, we found no statistically significant difference in adhesion formation between mechanical removal of the peritoneum and peritoneal destruction by minimal electrocoagulation.

These study's results suggest that superficial trauma to the peritoneum, either by mechanical denuding or minimal electrocoagulation does not necessarily lead to adhesion formation. However, trauma of the layers deep to the peritoneum—either by more severe electrocoagulation or by additional suturing through the underlying musculature—may lead to increased adhesion formation. This highlights the role of the tissue and musculature underlying the peritoneum in adhesion formation and suggests that the additive effect of suturing on adhesion formation, which is well established for mechanical traumatization of the peritoneum (9), also exists for trauma by electrocoagulation in this model.

Finally, the results of the current investigation suggest that the effect of bipolar electrocoagulation on adhesion formation might depend on the extent of coagulation. There appears to be a spectrum concerning the depth of trauma by electrocoagulation at the lower end of which there is little adhesion formation and the higher end, there is extensive adhesion formation.

A number of limitations should be taken into consideration when interpreting the current results. First, for the limited number of animals, the results of this study need to be verified in a larger study. Second, different areas were traumatized in different groups. However, with respect to the results, we feel confident to compare the groups and draw conclusions from that. In fact, the increased adhesion formation despite the smaller traumatized areas serves to highlight the important additive effect of suturing. Our results are unambiguous in demonstrating that minimal coagulation plus suturing leads to significantly more adhesions than minimal coagulation alone in this model. Third, the current investigation only considers the parietal peritoneum. These results need to be replicated for the visceral peritoneum and other tissues commonly traumatized during surgery, such as the ovary. Finally, in this study we took no specific action to keep the tissue moist during the intervention, although tissue desiccation may be one of the factors that leads to adhesion formation. Because the overall duration of the surgery was approximately 20 minutes and the differences in the duration between the groups were negligible, we are confident that tissue desiccation was not a confounding factor in this experiment. In future experiments tissue desiccation could be standardized between the groups by waiting a constant time before closure of the abdominal cavity.

In conclusion, superficial trauma, mostly limited to the parietal peritoneum, may be a negligible factor in adhesion formation. This appears to be irrespective of the mode of trauma. However, additional trauma of the underlying tissues, either by deeper electrocoagulation or suturing, leads to significantly increased adhesion formation. These data also show that there is a spectrum concerning the extent of trauma by electrocoagulation at the lower end of which there is little adhesion formation.

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Quantifying electrosurgery-induced thermal effects and damage to human tissue: An exploratory study using the fallopian tube as a novel in-vivo in-situ model

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Financial support:

Supported by the German Ministry of Education and Research (BMBF; Berlin, Germany), grant 16SV 1352 ("Minimally invasive technology and therapeutic methods"), and by a grant from the Research Foundation of the Department of Obstetrics and Gynaecology, University of Tübingen, Germany.

Previous presentation:

Presented as oral presentations at the 16th Annual Congress of the European Society of Gynecological Endoscopy (ESGE), in Portoroz, Slovenia, September 5–8, 2007 and at the 36th Annual Meeting of the American Association of Gynecological Laparoscopists in Washington, D.C., USA, November 14–17, 2007.

Précis:

Based on a scoring system involving deep-tissue and surface temperatures, gross assessment, and histology, a novel model allows standardized, reproducible, quantitative analysis of electrosurgery-induced thermal effects and damage to human tissue.

Abstract

Objective: To develop a human in-vivo in-situ model for analyzing the extent and the basic mechanisms of thermal spread and thermal tissue damage.

Design: Prospective, open, uncontrolled, non-randomized, single-center exploratory study.

Setting: University hospital.

Patient(s): Eighteen adult patients undergoing open abdominal hysterectomy for benign disease.

Intervention(s): Unilateral fallopian tube tissue desiccation (10 seconds) using a laparoscopic bipolar clamp at routine settings.

Main Outcome Measure(s): Deep tissue temperature (thermal probe), tissue surface temperature (thermal camera), and gross and histological assessments of lesions using a newly developed composite scoring system.

Result(s): Lateral thermal damage (LTD; determined by lactate dehydrogenase staining), was strongly correlated with maximum desiccation temperature. Deep tissue LTD and surface LTD were linearly related. Histological and macroscopic criteria for thermal effects and damage and the corresponding scores proved functional and strongly correlated with LTD. Measurement of deep tissue and tissue surface temperatures consistently yielded complete temporal and spatial temperature distributions that were describable by the heat equation.

Conclusion(s): Our novel in-vivo in-situ model allows standardized, reproducible, quantitative assessment of electrosurgery-induced thermal effects and damage in human tissue. It will likely provide further insight into the underlying biothermomechanics and may prove useful in the development of safety guidelines for laparoscopic electrosurgery.

Keywords: Surgical technique; bipolar coagulation; electrosurgery; thermal lesions; iatrogenic damage; tissue desiccation.

Introduction

Electrosurgery has contributed significantly to the success of laparoscopy as a therapeutic procedure since Kurt Semm performed the first laparoscopic appendectomy in 1983 (1). The advantages of laparoscopic surgery, which include shorter hospital stays, less blood loss and pain, and fewer post-operative adhesions (2), are largely due to constant technological improvements and innovations, not least the introduction of electrosurgical vessel sealing (3-5). Modern bipolar vessel sealing produces seals that withstand intraluminal pressures well above the physiological range (5-11) and in many cases is more efficient than other methods of ligation (e.g., suture, hemoclips, ultrasonic coagulating shears (UCS)) (7, 10).

However, energy-based vessel ligation is associated with complications, especially in the case of more complex laparoscopic interventions (3). Thermal injuries and ischemic injuries from direct heat exposure or thermal spread to adjacent tissues can induce hemorrhagic complications and tissue necrosis (4, 13, 14). This can lead to, e.g., bowel injury, a feared complication (4, 5) that may go unrecognized during

surgery and may not present until 3–14 days after surgery (6). Often, the mechanism of thermal complications is not understood and essentially it is unclear whether thermal spread, careless manipulation with heated devices, or heated tissue causes the reported complications, e.g., ureterovaginal, vesicovaginal or duodenal fistulas, and rectal perforations (15-17).

In response, numerous electrosurgical devices have entered clinical practice, including pulsed systems (10, 18, 19) or instruments with conductive paths inside their jaws (20). Many instrumental factors such as jaw size and clamp surface (7) or compressive pressure during sealing, maximum temperature and the dynamics of heat deposition into the tissue (8) have been found to be crucial to successful thermal fusion but they still await detailed investigation. Thus, to date, choice of technique appears to be based much more on surgeon preference than on objective human data. In particular, thermal spread and thermal tissue damage have so far only been investigated in animal studies based on postoperative histological analysis of vessel seal samples or observation of the extent of birefringence loss (11, 12), and although there are isolated reports of in-situ measurements in animals (9), detailed investigations in a human in-vivo and in-situ model are still lacking. Overall, the widespread clinical use of electrosurgery is not reflected in an equally detailed understanding of the underlying biothermomechanics (10) and the potential risks.

We therefore sought to establish a human in-vivo in-situ model for further analysis of the basic mechanisms of thermal spread and thermal tissue damage with a view to increasing the safety of laparoscopic surgery. For this purpose, we developed a model designed to generate comprehensive, easily reproducible, standardized data defining the thermal electrosurgery-induced effects on human tissue, based on four assessment categories: spatiotemporal changes in deep tissue temperature and tissue surface temperature; macroscopic scores; and microscopic scores.

Material and Methods

Study design. This was a prospective, open, uncontrolled, non-randomized, single-center exploratory study. The study protocol was approved in advance by the Ethics Committee of the Medical Faculty of the University of Tübingen, Germany as project number 425/2006M. Included were 18 patients older than 18 years who had already consented to, and were scheduled for, abdominal hysterectomy by laparotomy for benign disease. Patients were recruited consecutively, confirmed their ability and willingness to comply with study procedures and gave their written informed consent. All surgery was performed at the Department of Obstetrics and Gynaecology of the University of Tübingen, Tübingen, Germany. Work-up for surgery and general anesthesia during surgery were performed according to standard in-house procedures and documented in the patient records. Surgical exposure of the uterus and the fallopian tubes was performed in a routine fashion. The experimental interventions were carried out prior to resection of the fallopian tubes. The operation was then completed in the usual manner.

Intervention. Per patient, one fallopian tube was grasped and desiccated for 10 seconds with a laparoscopic bipolar clamp (Robi® Laparoscopic Forceps 38221 ON, KARL STORZ GmbH & Co. KG, Tuttlingen, Germany). The applied forceps pressure was standardized by a sprung handle. Energy was supplied by a VIO 300D electrosurgical unit (ESU; ERBE Elektromedizin GmbH, Tübingen, Germany) with the clinically established pulsed "Bipolar Soft" mode (automatically modulated sine-wave signal form with a fundamental frequency of 350 kHz as previously described (11) set to 40 Watts and Effect 5. Equipment, instruments and settings were routinely used for laparoscopic surgery in our hospital.

Temperature measurements. *Deep tissue temperature* was measured using four thermal probes (NiCr-Ni sheathed thermocouple assemblies (MTE) 1_R 9-13®, Reckmann Mess & Regeltechnik, Hagen, Germany) that were custom-built into standard 0.7 mm diameter needles. The probes were fixed to a linear guide rail and inserted directly into the center of the fallopian tube (Fig. 1A). Two probes were located at 4.5 mm and the other two at 11.5 mm to the left and right of the edges of the bipolar forceps (equivalent to 7 mm and 14 mm from the middle of the forceps). Deep tissue temperature was recorded at a rate of 1 Hz using Jumo Logoscreen CF equipment (Version 172.02.xx; JUMO, GmbH & Co. KG, Fulda, Germany) and the appropriate software for temperature monitoring and analysis (Version 2.06J). Prior to the study, the Safety Committee for medical equipment of the University of Tübingen confirmed that the use of the thermal probes raised no medical concerns according to the relevant standards, DIN VDE 0751-1 and DIN EN ISO 14791. Temperatures were recorded from 10 seconds before until 30 seconds after tissue desiccation was initiated.

Tissue surface temperature was studied using a precalibrated high-specification thermal imaging camera (VarioCAM® fitted with an LW IR 1.0/25 mm lens, JENOPTIK Laser, Optik, Systeme GmbH, Jena, Germany). The camera was set up outside the sterile surgical area on a tripod-mounted swing arm and positioned 0.5 m above the surgical field at an angle of 90° to the fallopian tube fixed in the rail. The equipment was operated by experienced users. Thermal images were taken on verbal command 1 second before and 1, 10, 20 and 30 seconds after the start of the tissue desiccation. The camera operated in the mid-infrared (8–13 µm) waveband and captured fully digitized 16-bit thermographic frames. Using the stored picture data, temperatures in each frame were later measured and analyzed with the IRBIS® Plus software (Version 2.2, InfraTec GmbH, Dresden, Germany).

Macroscopic analysis. As soon as the tube was resected, the desiccation site was described macroscopically in a quantitative, standardized fashion in consensus by two surgeons according to previously reported clinical criteria (7) (Table 1).

Histological analysis. The fallopian tube was cut longitudinally and stained using (a) hematoxylin and eosin and (b) the staining method for lactate dehydrogenase (LDH) activity described by Sherwood and Flotte (12). In LDH-stained slides, thermal artifacts such as the distance from the edge of the cauterized zone to the beginning of undamaged stroma or epithelium were measured both inside the tube, at the

epithelial level, and on the tubal surface. To avoid describing thermal artifacts such as homogenous darkly stained tissue, tissue fragmentation and epithelial destruction (13, 14) and streaming artifacts with smudged chromatin and elongated, hyperchromatic nuclei, and vacuolated signet cells of stromal derivation (15), we decided on the following four criteria for standardized and easily reproducible description of the microscopic changes: (1) epithelial alteration, (2) presence of tissue fragmentation as a differentiation between intact and detached internal mucosa (endosalpinx), (3) loss of histological tubal architecture due to tissue desiccation and (4) basophilia due to increased binding of hemalum following thermal damage. These criteria were assigned scores from 0 to 3 (Table 1). All microscopic slides were evaluated and scored in consensus by two different blinded observers.

Statistical Analysis: Data were analyzed using the R software, Version 2.7.2 (R Foundation for Statistical Computing, Vienna, Austria (16). The strength of relationship between two ordinal or continuous variables was estimated using Spearman's rank correlation coefficient r_s. The two methods for measuring temperature, i.e. thermal camera and thermal probes, were compared using the Bland-Altman method (17). The temperature distribution in space and time can be described by the heat equation. Therefore we expected that the one-dimensional temperature profile would roughly follow $b_1 \exp(-x^2/b_2)$, where x is the variable describing space, in our case the distance from the desiccation site. The parameters b_1 und b_2 were estimated by nonlinear regression. Estimation was done separately for each time point to allow for continued heating during the first 10 seconds.

Results

Complete data sets were recorded for 15 fallopian tubes from the 18 patients who underwent surgery. For 2 patients, fallopian tubes were not available because they were used to diagnose the underlying disease. In the third case, the thermal camera was not available. Table 2 details the diameter, maximum temperature, lateral thermal damage (measured microscopically), and the macroscopic and microscopic scores for each of the 15 specimens.

Maximum temperature, lateral thermal damage, and caliber of the fallopian tube: Maximum temperature rise (MTR) and mean lateral thermal damage (mLTD) as revealed by histology were strongly correlated ($r_s = 0.93$). Both MTR and mLTD demonstrated a moderate negative correlation with the tubal caliber (TC) ($r_s = -0.64$ for MTR and TC, and $r_s = -0.56$ for mLTD and TC). All other microscopic criteria were compared with the mLTD as the gold standard for the detection of thermal damage in this setting.

Deep tissue temperature: The data recorded at the center of the tube at 4.5 and 10.5 mm from the edge of the clamp are shown in Figure 2. The mean initial temperature (± SD; range) was 31.3°C (± 2.1°C; 28.1–34.3°C). At 4.5 mm distance, mean temperatures were > 60°C and > 50°C for > 4 seconds and 10

seconds, respectively. The mean MTR at 10.5 mm from the desiccation site was 5.0°C. For both distances, MTR was reached after 4 seconds.

Tissue surface temperature: The profile of surface temperature versus time and distance from the desiccation site is depicted in Figure 3. Mean initial temperature (± SD; range) was 27.9°C (± 2.2°C; 24.0–32.0°C). The curves demonstrate that the tissue closest to the desiccation site heated up first. Immediately after tissue desiccation (10 s), mean temperature at the desiccation site was approximately 85°C and did not drop below 50°C for the next 20 seconds.

Information value of thermal probe data vs. thermal camera measurements: The thermal probe and thermal imaging methods were compared for the temperature data measured at 4.5 and 10.5 mm from the edge of desiccation site at t = 1, 10, 20 and 30 seconds using the statistical methods described by Bland and Altman (17). Differences for mean values were increased, while relative differences remained approximately constant. For this reason, the Bland-Altman plot in Figure 4 shows log transformed data. On back transformation the temperature values recorded with the thermal camera were, on average, 102% of those obtained with the thermal probes (95% confidence interval of mean [99%; 105%]). Thus, camera-recorded temperatures were 2% higher on average, corresponding to an average 1°C difference between thermal camera and thermal probe measurements. However, the limits of agreement indicated that 95% of the camera-recorded temperatures could be expected to lie in an interval between 66% and > 158% of the temperatures measured with the thermal probes, corresponding to a difference range between −12°C and +18°C for the mean observed temperature values.

Histological analysis: A linear relationship was observed between internal and external LTD (R^2_{adj} = 0.82). The external LTD was 1.5 times greater than the internal LTD. All analyses reported below are based on mean LTD (mLTD), which was 2.34 mm (± 0.49 mm; range 1.6–3.4 mm).

The scores for epithelial alteration and tubal architecture were strongly correlated with mLTD (both r_s = 0.70), presence of tissue fragmentation was weakly correlated with mLTD (r_s = 0.22), and basophilia was moderately correlated with mLTD (r_s = 0.59). The sum score (Smi) for all 4 criteria was strongly correlated with mLTD (r_s = 0.82). Further analysis led to a simplified score (sSmi), the sum of epithelial alteration and tubal architecture, with r_s = 0.81, a correlation of similar strength as the one between Smi and mLTD.

Macroscopic analysis: Macroscopic criteria were also compared with the mLTD. The scores for tissue clarity and tissue desiccation were strongly correlated with mLDT (r_s = 0.76 and r_s = 0.82, respectively) while tissue charring and instrument sticking were both weakly correlated with mLTD (r_s = 0.29 and r_s = −0.27, respectively). The sum score (Sma) of all 4 criteria was strongly correlated with mLTD (r_s = 0.81). The best simplified score (sSma) was found to be the sum of the scores for tissue clarity and tissue

desiccation with a correlation coefficient of r_s = 0.83, which was as strong as correlation between Sma and mLTD.

Discussion

The objective of the present study was to establish an in-vivo in-situ model to study the thermal effects of electrosurgical tissue desiccation on human tissue and the damage it induces. There is a definite need for a standardized model, given the multitude of available electrosurgical devices, the unacceptably high number of thermal complications, and our deficits in understanding in detail the biothermomechanics of thermal fusion.

The ultimate endpoint of thermal damage is tissue necrosis. Histologically, thermal damage has always been described in a mostly dichotomous fashion and its detection and evaluation has reportedly been difficult (18) (19). To date, very few quantitative approaches have been pursued to evaluate thermal damage in a standardized yet detailed fashion (20). However, these were developed for skin lesions and were not suitable for our purposes. In the present study, we defined as our gold standard the assessment of mLTD as revealed by LDH staining according to Sherwood and Flotte (12). This method enables the detection of oxidative enzymes that correlate with cell function and activity and therefore is particularly suited to the assessment of thermally damaged tissue.

In our study, lateral thermal damage to fallopian tube tissue was very strongly correlated with the maximum temperature rise within the tissue, which is in accordance with theoretical models of thermal fusion (21) and aspects supporting LDH staining. Both parameters demonstrated a moderate negative correlation with tubal caliber, which suggests that heat development decreases with increasing tissue volume, thus limiting heat development and hence, to a certain degree, thermal damage. Interestingly, the thermal damage measured on the surface of the fallopian tube was approximately 1.5 times higher than at the center of the tube, indicating that the lateral spread of the thermal lesion was greater than its depth of penetration. One explanation for this phenomenon could be that the fluids on the surface and in the outer layers of the tissue heat up and evaporate more quickly than those in the deeper layers, thus adding an exogenous heat component to the endogenous heat effect through the release of energy as dictated by Joule's Law.

In tissue desiccation, tissue response is governed by the amount of heat and the dynamics of heat deposition, in conjunction with the tissue properties and mechanical parameters (8, 13). Thus, temperature is a key factor in thermal fusion as well as thermal damage. In our study, mean surface temperature at the desiccation site was approximately 85°C immediately after dessication and did not drop below 50°C during the next 20 seconds. Mean deep-tissue temperatures at 4.5 mm lateral to the desiccation site did not exceed 50°C for more than 10 seconds. The resulting mean LTD measured 2.3 mm, which is consistent with literature reports that the minimum temperature for tissue dessication is

50°C (22, 23) and that thermotherapy requires temperatures of about 50°C or 55°C to be maintained for at least 15 seconds (22, 24).

Both thermal probes and thermal cameras generate valuable information on the changes in deep-tissue and surface temperature in space and time. However, the data obtained with these two modalities differ considerably. A small portion of the differences can be explained by the observation that the spread of thermal damage is greater on the surface than within the tissue because the heating process produces higher temperatures on the tissue surface. This interpretation is supported by our finding that mean temperatures were 1°C higher for the thermal camera compared with the thermal probes. Until such time as this has been studied in greater detail, it seems best to combine the two modalities to obtain comprehensive and accurate temperature data.

Based on previous reports of thermal artifacts in the fallopian tube and the cervix (13-15), we devised a histological score Smi consisting of the four criteria epithelial alteration, tissue fragmentation, tubal architecture and basophilia. In our present study, Smi was strongly correlated with mLTD. Therefore, our composite score and its individual criteria were, in combination with LTD detection, suited to assessing the effects of tissue desiccation in a more quantitative manner, thus rendering them more comparable. Detailed, reliable histological evaluation is particularly important in this model since lateral spread as assessed by histology is less pronounced than indicated by real-time thermography. This is because the extent of permanent damage depends not only on maximum temperature but also on the duration of heat application (9). Moreover, our score could be useful as a tool to compare electrosurgery with other modalities with regard to collateral damage, e.g., ultrasonically induced proximity damage, which is not easily detected macroscopically (25). Although it appears that a simplified score, sSmi, comprising only two criteria – epithelial alteration and loss of histological tubal architecture – adequately reflects the induced histological changes, further studies will be needed to confirm or refute this finding.

In clinical routine, thermal effects and even injury are often only assessed macroscopically. As with histological evaluation, there is no established standardized quantitative protocol. We therefore considered it necessary to include macroscopic analysis in our model as a 4^{th} category. The criteria constituting the macroscopic score were available from the literature (7) but had not been evaluated with regard to their ability to reflect tissue damage as defined by histology. In our present study, the sum score, Sma, was found to reflect the histology of our specimens quite accurately. The simplified score, sSma, representing the sum of only the two strongly correlated criteria, produced equally good results and can be considered sufficient for macroscopic analyses.

Our model as reported here admittedly leaves room for improvement. Firstly, maximum temperature rise as suggested by the thermal probe data was reached after about 4 seconds, a time at which no thermal images were taken. Secondly, all thermal images were taken on verbal command, which may have led to some delay. These issues will be resolved in future studies by using automated thermal imaging at a rate

of 1 Hz. Moreover, the innermost thermal probes will be positioned much closer (2 mm instead of 4.5 mm) to edge of the clamp than in the present study so as to capture the details of the temperature dynamics more accurately and avoid missing most of the temperature dynamics. Finally, the exact position of the camera is a compromise between adequate spatial resolution and obstruction of the surgeon's view and range of action. The low standard deviations of our measurements, however, demonstrate that the task is feasible and reproducible.

In summary, we consider that our model produces comprehensive and easily reproducible data in a standardized manner suitable for the evaluation of thermal effects and damage to human tissue. In combination, the tissue surface and deep tissue temperature measurements enable a more in-depth analysis of tissue temperature profiles and their dynamics than either method by itself. Quantitative macroscopic and microscopic evaluations of thermal damage based on multiple criteria allow the thermal effects of various electrosurgical instruments to be compared at different tissue desiccation settings. Consolidated evaluation of temperature and thermal damage enables investigators to better understand, and also quantify, the relationship between heat and tissue damage.

Conclusions

For the first time, in-vivo in-situ real-time temperature measurements in humans have been combined with thermography, macroscopic analysis, and histology to establish a standardized model for further investigation of the thermal effects and damage induced by electrosurgical tissue desiccation. Understanding the underlying biothermomechanics will help to develop safety guidelines for the handling of electrosurgical instruments in laparoscopic surgery.

Acknowledgements

We thank Dr. Peter Fritz and Dr. Thomas J. Flotte for histological analysis, Dr. Markus Enderle and Dipl.-Ing. Daniel Schäller for technical assistance and Dr. Birgitt Schönfisch for support with the statistical analysis.

Tables

Table 1

Macroscopic and microscopic scores.

Evaluation	Criteria	Score
Macroscopic*	Tissue clarity	0 = not translucent; 1 = slightly translucent; 2 = moderately translucent; 3= fully translucent
	Tissue desiccation	0 = wet; 1 = slightly dried; 2 = moderately dried; 3 = fully dried
	Tissue charring	0 = no charring; 1 = few black spots; 2 = confluent black spots; 3 = completely black tissue area
	Instrument sticking	0 = no sticking; 1 = sticking but easy to remove instrument; 2 = sticking and difficult to remove instrument
Microscopic	Epithelial alteration	0 = none; 1 = some; 2 = moderately prevalent; 3 = frequent
	Tissue fragmentation	0 = none; 1 = some; 2 = moderately prevalent; 3 = frequent
	Tubal architecture	0 = no loss of architecture; 1 = loss of separation between endosalpinx and myosalpinx; 2 = inner circular and outer longitudinal myosalpinx undiscernible; 3 = complete loss of structure in endosalpinx, myosalpinx and serosa
	Basophilia	0 = no basophilia; 1 = slight basophilia; 2 = moderate basophilia; 3 = marked basophilia

* based on (7)

Table 2

Diameter, maximum temperature, lateral thermal damage, macroscopic and microscopic scores for each fallopian tube specimen.

			Macroscopic Scores				**Microscopic Scores**						
							Lateral thermal damage (LDH staining in mm)						
Specimen	**Diameter** (mm)	ΔT_{max} (°C)	*Tissue clarity*	*Tissue desiccation*	*Tissue charring*	*Instrument sticking*	*Center of tube*	*Surface of tube*	*Mean*	*Epithelial alteration (0–3)*	*Tissue fragmentation (0–3)*	*Tubal architecture (0–3)*	*Basophilia (0–3)*
1	6.0	53	1	2	1	1	1.8	2.4	2.1	2	1	1	1
2	6.0	55	1	2	1	1	1.7	2.6	2.2	1	2	2	2
3	4.0	71	2	3	1	0	2.4	3.4	2.9	3	1	3	3
4	4.0	50	0	1	1	1	1.3	2.1	1.7	1	1	1	2
5	5.0	58	1	2	3	0	1.8	3.0	2.4	3	1	2	1
6	3.5	64	2	3	3	0	2.2	3.4	2.8	2	2	3	2
7	5.0	54	0	2	1	0	1.7	2.3	2.0	2	2	2	2
8	4.0	79	2	3	1	0	2.6	4.1	3.4	3	2	3	3
9	5.5	46	0	1	1	0	1.4	1.7	1.6	1	0	2	1
10	5.0	59	0	2	1	0	1.9	3.3	2.6	2	0	3	3
11	5.0	60	2	3	1	0	2.0	3.0	2.5	2	2	2	3
12	6.0	54	1	2	1	0	1.6	2.3	2.0	2	2	2	2
13	4.5	59	2	2	2	0	2.1	3.4	2.8	3	1	3	2
14	6.5	50	1	1	1	0	1.5	2.5	2.0	2	1	1	1
15	6.5	53	0	2	2	0	1.5	2.6	2.1	1	0	3	2

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Figures

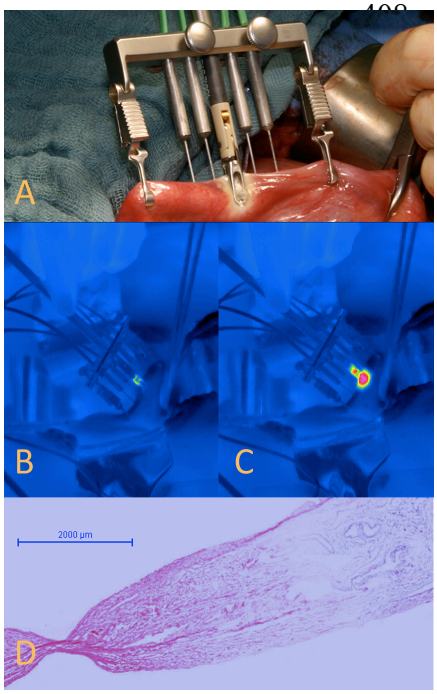

Figure 1

Experimental setup and tissue specimen: (A) tissue desiccation device comprising a bipolar clamp (center) and two lateral pairs of temperature probes; images taken with the thermal camera at the beginning (B) and during (C) tissue desiccation; and (D) LDH activity in a desiccated fallopian tube.

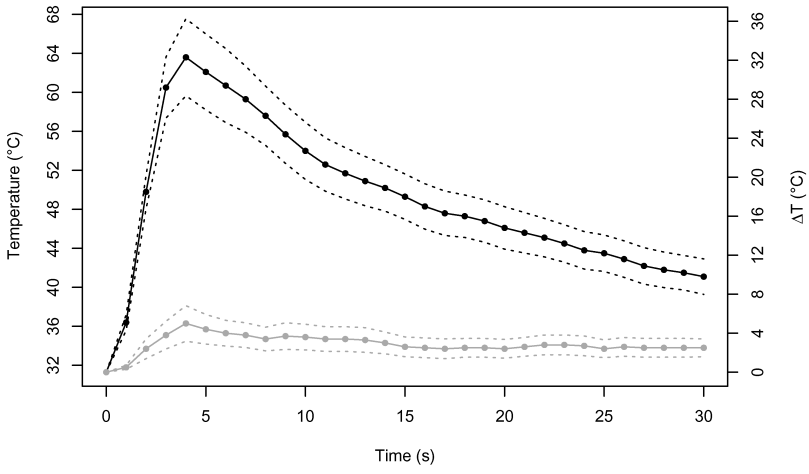

Figure 2

Mean temperature (left y-axis label) and mean temperature rise ΔT (right y-axis label) over time with 95% confidence intervals (dashed lines) at 4.5 mm (black dots) and 11.5 mm (grey dots) from the edge of the desiccation site.

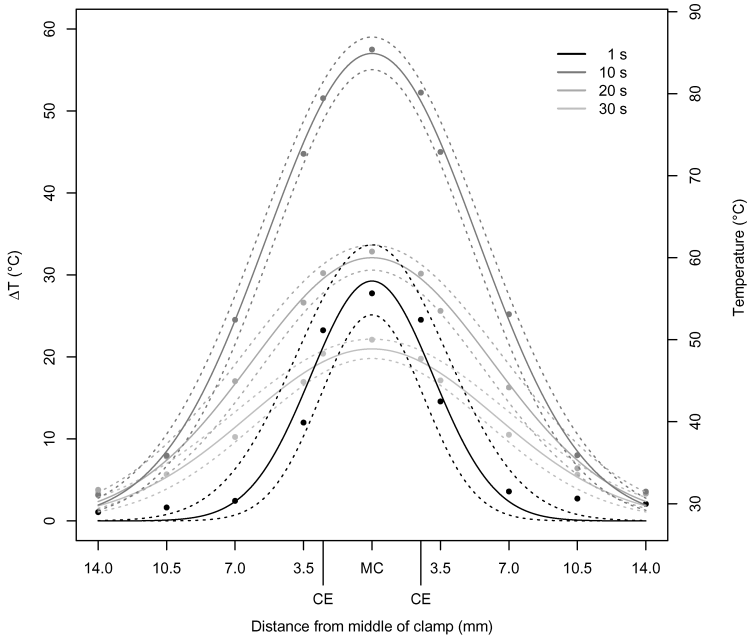

Figure 3

Distribution of mean surface temperature rise as a function of distance from the desiccation site, shown as data points (dots) and fitted curves (solid lines) with 95% confidence interval curves (dotted lines). MC = middle of clamp, CE = edge of clamp (= 2.5 mm from the middle of the clamp). Points and curves represent data at 1, 10, 20 and 30 seconds (black, dark grey, grey, and light grey, respectively).

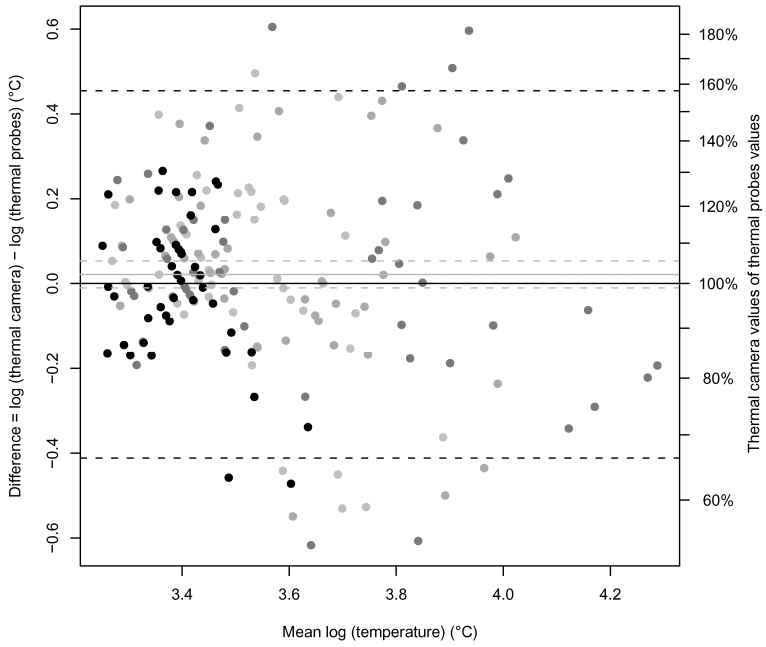

Figure 4: Bland-Altman plot of log transformed temperature data recorded with a thermal camera and with thermal probes. Differences at 1, 10, 20 and 30 s are plotted as dots (black to lightgrey). Also shown are the mean difference of logs (grey solid line) with 95% confidence intervals (grey dashed lines) and limits of agreement (black dashed lines).