

Szent István University
Postgraduate School of Veterinary Science

**Studies on new electrosurgical techniques:
the EnSeal[®] vessel- and tissue-sealing system**

Theses of PhD Dissertation
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CONTENTS

1. INTRODUCTION	3
2. STUDIES ON THE ENSEAL[®] ELECTROSURGICAL VESSEL- AND TISSUE-SEALING SYSTEM	5
2.1 Materials and methods	6
2.2 Results and discussion	8
3. USE OF ENSEAL[®] FOR LAPAROSCOPIC SURGICAL INTERVENTIONS ON STANDING HORSES	12
3.1 Materials and methods	12
3.2 Results and discussion	13
4. USE OF ENSEAL[®] FOR DIFFERENT TYPES OF SOFT TISSUE SURGERY IN DOGS	14
4.1 Materials and methods	14
4.2 Results and discussion	14
5. NEW SCIENTIFIC RESULTS	19
6. PUBLICATIONS REPORTING THE RESULTS OF THE POSTGRADUATE RESEARCH	21
7. ACKNOWLEDGEMENTS	23

1. INTRODUCTION

Earlier, we had used exclusively conventional surgical techniques at our University Clinics (at the Clinic of Surgery and Ophthalmology of the Faculty of Veterinary Science, Szent István University and at the Large Animal Clinic in Üllő). We had performed abdominal and thoracic surgeries via laparotomy and thoracotomy, and achieved haemostasis by the use of different surgical suturing materials, clamps and standard electro-surgical devices. However, in recent years it has become possible to perform minimally invasive surgical interventions (laparoscopy, thoracoscopy) and electro-surgical vessel and tissue sealing by the use of the EnSeal[®] system available in the framework of my PhD work. EnSeal[®] belongs to the group of vessel- and tissue-sealing devices within the class of electro-surgical instruments included in the larger category of electromagnetic energy-based surgical instruments. In tissues treated with this device, the high pressure and the controlled temperature increase lead to the molecular transformation of collagen present in the tissues, resulting in an irreversible occlusion of the lumen of blood vessels; thus, haemostasis is achieved without the need to implant extraneous materials.

In the first period of my work, I performed a detailed search of the special literature regarding the properties of electromagnetic energy-based surgical instruments, as the safe use of such devices requires a thorough knowledge of the physicochemical and technological background and the limitations and risks of procedures involving their use. Relatively few data are available in the literature about the properties of EnSeal[®], and even the data that do exist lack consistency. Therefore, in the preclinical phase my objective was to standardise the testing methods and to evaluate the properties of the device in a quantitative manner. We conducted safety tests on pigs and their different tissues. We studied the burst pressure of the sealed vessel segments, detected temperature changes in the treated tissues with a thermocamera, and evaluated the dimensions of the microscopic thermal injury zone in the surrounding tissues by the use of conventional histological procedures and the NBTC enzyme histochemical technique. The clinical application of the device was started only after the favourable results of the above-mentioned studies had become available.

In the clinical phase, my objective was to use, combine and evaluate the new techniques and to extend the potential fields of use of the EnSeal[®] device. We started the clinical application of the EnSeal[®] instrument at the Large Animal Clinic, for laparoscopic cryptorchidectomy and ovariectomy in sedated standing horses, then continued its use for different conventional and

minimally invasive soft tissue operations on dogs in the small animal operating theatre block of the Clinic of Surgery. During these operations, we consistently used the EnSeal[®] adaptive bipolar electro-surgical vessel- and tissue-sealing device for sealing and cutting through of the blood vessels and tissue connections of the structures to be removed. The device proved to be safe and effective for all types of surgeries tested, on all occasions.

2. STUDIES ON THE ENSEAL[®] ELECTROSURGICAL VESSEL- AND TISSUE-SEALING SYSTEM

Due to its numerous favourable features, EnSeal[®] stands out from the group of electrosurgical tissue-sealing devices. The most important positive properties to be mentioned are the Positive Temperature Coefficient (PTC) technology and unique electrode configuration of the handpiece (Smart Electrode), the built-in cutting blade (I-Blade) and the advantages offered by these features.

The dedicated generator of EnSeal[®] is the RF-60 generator, but the device can also be used with the Erbe Vio[®] electrosurgical units and the Ethicon G11 generator. The radiofrequency energy provided and monitored by the generator is conveyed to the patient's tissues by the EnSeal[®] bipolar handpiece. The handpiece can be used during both conventional and minimally invasive surgical interventions. The standard handpiece has a pistol-grip handle, a 140-, 250-, 350- or 450-mm-long connecting shaft 5 mm in diameter, and 20-mm-long forceps-like jaws that may be rounded or angular on one side. The shaft diameter of the EnSeal[®] Trio handpiece is also 5 mm, but the curved jaw of this instrument is only 3 mm in diameter. The newest member of the product range is the EnSeal[®] G2 Superjaw, which has 220 mm shaft length, 12 mm shaft diameter, 40 mm jaw length and 6.7 mm jaw diameter. The movable grasping surfaces of the handpiece contain a matrix of several hundred thousand nanoparticles operating as distinct thermosensitive control points (thermostats). If during the activation process the temperature does not yet reach the critical value (100 °C) at a given point of the matrix, the nanoparticles will be arranged in a chain, thus enabling the energy flow and the temperature elevation. If the temperature rises above the critical value (100 °C) at the given matrix point, the particles will assume an amorphous arrangement, and local heat transfer will stop. This process is called pulsating energy transfer. The instrument applies this special control until the tissues grasped between the jaws are homogeneously sealed, independently of the quality and quantity of their elements. When tissue sealing is completed (after a few seconds) the process will stop automatically, independently of the operator, and this is indicated by a long audio signal (a whistling sound). Owing to the unique electrode configuration, the collateral thermal injury of the tissues and the smoke generation are minimal. The collagen elements of the blood vessels and tissues to be sealed provide the natural sealant for the tissue-sealing process, which occurs as a result of the structural transformation of proteins brought about by the effect of elevated temperature and the strong compression exerted by the I-blade. The sealed vascular stumps can resist even

supraphysiological pressures. As with the use of this procedure, unlike the electrosurgical procedures applied earlier, the lumen of blood vessels is not closed by a thrombus, no subsequent detachment or postoperative bleeding needs to be reckoned with after the cessation of angiospasm or the postoperative elevation of blood pressure. Thanks to its atraumatic teeth, the jaws of the handpiece are suitable for grabbing and manipulating the tissues and, with the help of the I-blade that can be pushed forward, also for dissection. Unlike with the previously used systems, sealing and dissection can be done in one step, without moving the instrument, which greatly increases the safety of dissection (it is quite certain that the cut occurs exactly in the middle of the sealed area) and makes it redundant to use additional cutting devices.

The LigaSure™ instrument, which is currently a market leader in the field of electrosurgical vessel- and tissue-sealing systems, appeared on the market 5 years before the EnSeal® device. The special literature dealing with it has grown to a substantial size, while relatively few data are available about the EnSeal® system. Although the authors of publications presumably made attempts to standardise their testing methods and thus render their findings comparable, the results are still rather inconsistent.

My objective was to standardise the testing methods and then to study and quantitatively evaluate the parameters of the EnSeal® device. The successful sealing of the treated blood vessels or tissue bundles and their resistance to supraphysiological pressures (positive effect) was regarded as the first safety issue, and the degree of undesirable tissue injury caused by collateral heat generation (negative effect) as the second safety issue.

2.1 Materials and methods

During our own investigations, like in the overwhelming majority of studies reported in the literature, we used samples obtained from experimental pigs. The blood vessel- and tissue-sealing procedures and the subsequent samplings were performed under general anaesthesia.

For the burst pressure measurements we sealed and removed 60 different blood vessel segments. The samples were 3–4 cm long, non-branching, closed at one end and open at the other. Based upon their *in vivo* diameter, we divided the blood vessel segments into three groups: 2–3 mm, 4–5 mm and 6–7 mm. Each group contained 10 arteries and 10 veins. The EnSeal® device is rated to seal vessels up to 7 mm in diameter, but as its 20-mm-long jaws

can accommodate vessels up to 12 mm in diameter, we studied also blood vessels 8–12 mm in diameter. After preparation of the vessel segments, we infused physiological saline of body temperature into their lumen, which resulted in gradually increasing pressure. The burst pressure values were recorded with a digital manometer. Since during the clinical interventions it is impossible to determine each of the parameters affecting the efficacy of sealing (type, size and structure of the blood vessels and their different collagen content), but as the sealing of all vascular structures must surely be accomplished (although the venous blood pressure is much lower), as a requirement we uniformly designated a burst pressure of 360 mmHg, i.e. three times the mean systolic blood pressure in humans.

Heat generation and thermal spread were recorded with a thermocamera during the sealing of 22 mesenteric blood vessels (jejunal arteries and veins) after median laparotomy. On the display of the thermocamera the temperature scale, the pixel temperatures, the minimum and maximum temperatures and the isothermal lines appeared in real time. The camera recorded the raw thermographic data which were subsequently evaluated with the help of software programmes belonging to the device.

Samples taken from the treated tissues (liver, spleen, abdominal wall muscles, different blood vessels in the splenic, gastric and mesenteric area, jugular vein, carotid artery) were preserved in 8% buffered formaldehyde solution, processed, embedded in paraffin, sectioned and stained with haematoxylin and eosin. Additional sections were stained with Giemsa, picosirius red and Heidenhain's azan stain, resorcin-fuchsin staining, Gömöri's hexamethylenetetramine silver staining procedure and the periodic acid–Schiff (PAS) reaction.

Further tissue samples (N=183; 30 liver, 6 spleen, 21 abdominal wall muscle samples and 126 blood vessels, of which 33, 24 and 42 originated from the splenic, gastric and mesenteric area, respectively, while 15 were derived from the carotid artery and 12 from the jugular vein) were processed by enzyme histochemistry. The fresh samples were quick-frozen and divided into further two groups that were stored at $-20\text{ }^{\circ}\text{C}$ and $-80\text{ }^{\circ}\text{C}$, respectively, until processed and then sectioned with a cryostat. For the enzyme histochemical tests, the nitroblue-tetrazolium chloride (NBTC) reagent was used. To measure the extent of collateral thermal injury in the sections, we used a Mirax Midi virtual microscope as well as a SPOT Xplorer digital camera and SPOT Advanced software connected to an Olympus BX-60 microscope. The requirement set by us was that following the use of the EnSeal[®] device the total microscopic thermal injury zone (MTZ_t), i.e. the entire dimension of bilateral tissue injury perpendicular to the longitudinal axis, should be <7 mm in

diameter, and the collateral microscopic thermal injury zone (MTZ_{lat}), i.e. the diameter of the unilateral tissue injury, should be <1 mm.

The results of the measurements were processed with the R (2010) statistical software. The differences were considered significant at $p < 0.05$. The results of burst pressure measurements and the MTZ_t and MTZ_{lat} values were evaluated by Student's one-sample *t*-test, while the mean values of the sample groups were compared by analysis of variance (ANOVA). The pressure, temperature and length values were expressed as mean \pm standard error of the mean (minimum value – maximum value).

2.2 Results and discussion

All of the vascular segments tested (N=60) remained intact at an intraluminal pressure increased to 360 mmHg, and thus the burst pressure significantly (Student's one-sample *t*-test) increased the designated limit. Bursting occurred at an average pressure value of 873.89 ± 120.57 (425–1555) mmHg, and typically not in the area of sealing. The few porcine vessel segments 12 mm in diameter tested by us also resisted the pressure of 360 mmHg. The highest recorded pressure, measured in a porcine carotid artery sample 5 mm in diameter, was 1555 mmHg.

Our thermocamera recordings clearly illustrate the temperature dynamics shown by EnSeal[®] and the surface of the tissues treated. The temperature cross-section of the activated handpiece can be characterised by a bell-shaped curve and a W-shaped thermal shadow at the peak of the former. The three temperature peaks occurred at the edges of the jaws and on the longitudinal axis and, owing to the design of the handpiece, they remained substantially below the maximum temperature (100 °C) of the tissues caught between the jaws of the device. In the case of the mesenteric blood vessels examined, the surface temperature of the tissues at the edge of the jaws increased to an average value of 69.25 ± 0.98 °C during activation. At the end of activation, immediately after removing the device, the average temperature measured at the surface of the tissues was 66.89 ± 0.66 °C. The average temperature decrease of the tissues was 14.50 ± 2.97 °C in the first 4 seconds and further 3.90 ± 0.42 °C in the subsequent 4 seconds after inactivation, and after the 10th second the temperature fell below 40 °C in all cases. The temperature of the examined tissues in the period from one second before to the 10th second after the end of activation can be described with the following polynomial equation: $y(^{\circ}\text{C}) = -0.0139*(x_{(s)}+2)^4 + 0.3284*(x_{(s)}+2)^3 - 2.3627*(x_{(s)}+2)^2 + 2.6372*(x_{(s)}+2) + 68.661$. The temperature of the

handpiece shaft remained below 40 °C throughout the interventions in all cases, but the surface temperature of the jaws followed the temperature changes of the tissues. Multiple consecutive activations resulted in a more lasting temperature elevation. It should be mentioned that during the clinical interventions the position of the jaws had to be controlled continuously until they cooled off, in order to avoid iatrogenic thermal injury. The thermal zone examined with the thermocamera and the thermal injury zone evaluated later in histological sections proved to have similar dimensions; however, the relatively low resolution of the thermocamera recordings did not allow us to achieve the high measurement accuracy set as a target.

The degree of thermal injury was closely correlated with the maximum temperature but it also depended on the duration of treatment, the interval between two treatments, the perfusion (cooling effect) and other biothermomechanical properties of the tissue treated. Whitish discolouration of the treated tissues (due to the denaturation of proteins) and transparency of the sealed blood vessels could be observed also macroscopically in all cases; however, the accuracy of measuring the dimensions of the macroscopic change failed to reach the level expected by us. We used histological methods for evaluating the thermal injury. During the light microscopic examination of histopathological sections stained with haematoxylin and eosin we found that the lumen of blood vessels in the treated tissues had become occluded and the vascular wall had become homogenised, denatured and hypereosinophilic. The nuclei of smooth muscle cells of the *tunica media* had undergone fragmentation, assumed a corkscrew-like, elongated shape or became pycnotic, and in some cases they contained vacuoles. The fine structure of the denatured collagenic fibres disappeared and became homogenised. The *lamina elastica interna* of the blood vessels could not be found or was fragmented, and in its microenvironment tissue fissures could be observed. A small number of activated neutrophilic granulocytes could be found in the affected areas. In areas more distant from the intervention the integrity of the stroma, its cells and intercellular constituents was unchanged. On the basis of our studies it can be established that the haematoxylin and eosin staining and – in different tissues and to varying extents – all the other classical staining procedures applied by us are suitable for the qualitative evaluation of thermal injury; however, the gradual transition between the thermally injured tissue and the neighbouring intact tissue areas does not allow a sufficiently high-precision quantitative evaluation of the MTZ.

Polarisation microscopy can detect the fine structural changes of collagen already above a temperature of 54 °C, but the transformation necessary for blood vessel sealing starts only at a temperature of 62–67 °C and lasts until a temperature of 95–100 °C is attained. During our

enzyme histochemical studies we evaluated the activity of the LDH enzyme. This enzyme proved to be active at a temperature below 64 °C and produced a bluish-violet formazan precipitate from the NBTC reagent. At temperatures exceeding 64 °C LDH was inactivated and did not produce a colour reaction visible in light microscopic sections. Inactivation of the LDH enzyme at 64 °C can be confirmed by the staining of samples incubated in water-baths of different temperature. The sharp demarcation line visible on the sections corresponds to the 64 °C isothermal line, i.e. the line formed by points of identical temperature, which is also an indicator of incipient collagen denaturation. During the freezing of LDH-containing tissues the enzyme maintained its activity, as no difference was found in colour reaction between samples stored at –20 °C and at –80 °C.

The resolution of the Mirax Midi virtual microscope proved to be unnecessarily high (the size of a single digitised image was several hundred megabytes), and therefore we decided against using this testing method. The group of samples processed by the enzyme histochemical method as well as the SPOT Xplorer camera and SPOT Advanced software connected to the Olympus BX-60 microscope were found to be suitable for quantitative evaluation. The MTZ values measured on the basis of colour intensity differences are of microscopic precision and reproducible, and subjective intra- and interobserver errors do not occur. Calculated for all samples, the average MTZ_t value was 5.55 ± 0.08 mm and, within that, the average MTZ_{lat} value was 0.28 ± 0.04 mm. Thus, the MTZ_t value was significantly below the expected 7 mm limit and the MTZ_{lat} value was significantly lower than the required 1 mm limit. The differences among the different sample groups, tissues and blood vessels in terms of their MTZ values were not significant and, thus, they could be considered statistically equivalent (ANOVA). The adequate perfusion of the *in vivo* tissues (intraoperative blood pressure control, infusion therapy), the cooling/heat-exchanging effect of blood circulation and of the turbulent blood flow developing after sealing, and the cooling effect of ambient temperature (open abdominal cavity) probably influenced the values obtained in a favourable manner. In tissues with poor or no perfusion and during laparoscopic interventions a wider thermal injury zone is likely to develop.

In our opinion, the NBTC enzyme histochemical procedure is suitable and can be recommended for future high-accuracy standardised comparisons of thermal injury induced by different performance levels and application times of various electromagnetic energy-based instruments (not only the blood vessel- and tissue-sealing devices) in different tissues. For the standardisation of burst pressure measurements, we recommend to use pig-derived non-branching, fresh arteries and veins of similar length (3–4 cm) and grouped according to

their diameter (2–3, 4–5, 6–7 and >7 mm), as well as a limit value of 360 mmHg (three times the average systolic blood pressure in humans).

3. USE OF ENSEAL[®] FOR LAPAROSCOPIC SURGICAL INTERVENTIONS ON STANDING HORSES

My objective with the following series of cases was to extend the clinical use of the EnSeal[®] device and to use, combine and evaluate novel surgical solutions surpassing the conventional operative techniques. Thus, the horses were standing rather than positioned in dorsal recumbency, only sedation was used instead of general anaesthesia, laparotomy was replaced by a minimally invasive laparoscopic intervention, and haemostasis was in all cases achieved by the use of the tissue-sealing device EnSeal[®] rather than by implanting extraneous ligating materials.

3.1 Materials and methods

The studies were conducted at the Large Animal Clinic of the Faculty of Veterinary Science, Szent István University in Üllő. Eleven horses (8 stallions and 3 mares) were included in the study. On these 11 patients, 8 unilateral cryptorchidectomies and 4 ovariectomies were performed. As premedication, detomidine or xylazine followed by butorphanol was applied. The sedated state of standing horses was maintained by the intravenous administration of detomidine and butorphanol. An Olympus laparoscopic system was used for the surgical interventions. Through one of the portals we introduced the EnSeal[®] tissue-sealing device and used it for the step-by-step sealing of blood vessels supplying the testis and epididymis or the ovary, and for transecting the associated soft tissues (the mesorchium or the mesovarium, mesosalpynx and proper ligament of the ovary). Through another portal we introduced a laparoscopic manipulator, which was used for keeping in place the testis and epididymis and the ovary during the tissue-sealing process and for removing these organs from the abdominal cavity thereafter. The patients remained under supervision in the hospital for 2–3 days after the operation. The removed tissues were preserved in 8% buffered formaldehyde solution. After preparation and processing, the sections were stained with haematoxylin and eosin and used for examining the histomorphological parameters of the lesions.

3.2 Results and discussion

In all cases, the EnSeal[®] device was used successfully for grabbing and separating blood vessels and soft tissues as well as for the safe sealing and dissection of blood vessels and tissue bundles. As blood vessel and tissue sealing and the dissection of tissues can be accomplished in a single step, without moving the EnSeal[®] device, the sealed tissue was cut in all cases and bleeding did not occur. We activated the electrosurgery device only if the device, the target organ and its surroundings were clearly visible and if the device came into contact only with the target organ, and thus undesirable thermal injury did not occur in any case. The generated amount of surgical smoke was minimal and it did not interfere with visualisation. The procedure did not require the introduction of extraneous material and it shortened the operative time. During the individual surgical interventions the device had to be activated 5–7 times, and the sealing and dissection of each vessel and tissue bundle caught between the jaws of the device, depending on its thickness and structure, required 2–8 seconds. The total operative time (from the start of insufflation until the placement of the last skin suture) was on average 82 min, while the net operative time (the actual surgical manipulation) was 30–40 min, and both values tended to decrease as we approached the end of the series of operations.

After the operations, the patients' condition was monitored for at least one month. During that period, no complication attributable to the intervention occurred. The owners were satisfied with the therapeutic and cosmetic results of the operations.

By histological examination, the unilateral ovarian neoplasms proved to be granulosa cell tumours, and the cryptorchid testes were devoid of neoplastic lesions. Gradual separation of the intact tissue from the treated, compacted, dehydrated and homogenised tissue areas and occlusion of the lumen of blood vessels treated with the tissue-sealing device could be observed in all histological sections.

During the interventions performed by us, we successfully combined the advantages of sedation, laparoscopy and tissue sealing. We successfully applied the EnSeal[®] device in all cases of laparoscopic cryptorchidectomy and ovariectomy performed on sedated horses in standing position. We were the first to report such operations in the special literature. On the basis of our results, we recommend the optional use of the EnSeal[®] device for the removal of intra-abdominal cryptorchid testes and ovaries in horses.

4. USE OF ENSEAL[®] FOR DIFFERENT TYPES OF SOFT TISSUE SURGERY IN DOGS

My objective was to extend the field of use of the EnSeal[®] tissue-sealing system and to evaluate the benefits derived from its use for different types of soft tissue surgery in dogs. The interventions described below were performed on dogs under general anaesthesia. The thoracic and certain abdominal operations were carried out in a minimally invasive manner (laparoscopy, thoracoscopy), and haemostasis was achieved with the help of EnSeal[®] in all cases.

4.1 Materials and methods

At the Clinic of Surgery and Ophthalmology of the Faculty of Veterinary Science, Szent István University, we used the EnSeal[®] tissue-sealing system for sealing and dissecting different blood vessels and tissue bundles during operations on 62 dogs. We performed 40 ovariectomies, 5 cryptorchidectomies and 4 liver biopsies by laparoscopy, 4 splenectomies, 2 mesenteric skeletisations and 1 partial pancreatectomy by laparotomy, and 1 lung biopsy and 2 partial pericardiectomies by thoracoscopy. In the case of three patients unilateral removal of the eyeball (enucleation) was done.

The examinations, the preparation of patients and the anaesthesia were done according to the general protocol of the clinic. We used the Storz endoscopy system of the Clinic for the minimally intensive interventions and standard surgical instrument kits for the conventional operations. Sealing and dissection of the designated blood vessels and tissue bundles were done with the EnSeal[®] tissue-sealing system in all cases.

4.2 Results and discussion

The overwhelming majority of the surgical interventions (40/62) were laparoscopic ovariectomies performed on healthy patients for neutering purposes. The patients were positioned in dorsal recumbency, in Trendelenburg position. After the induction of pneumoperitoneum (13 mmHg intra-abdominal pressure) the optical unit was positioned in the median line, 1–2 centimetres caudal to the umbilicus. The portal was installed in the

midline, at the midpoint of the distance between the umbilicus and the pubic bone. When using a single portal, the ovary was lifted to the abdominal wall with a manipulator and temporarily secured there with percutaneous transabdominal sutures, then separated it with the help of the EnSeal® device without any bleeding and removed it with the manipulator through the abdominal wall wound of the portal. If two portals were used, they were created at the midpoint of the distance between the umbilicus and the pubic bone, slightly lateral to the rectus abdominis muscle. Through one of the portals, we introduced the EnSeal® device and used it for sealing the blood vessels supplying the ovary and for sealing and dissection of the soft tissues connected to it. Through the other portal, we fixed the ovaries with the laparoscopic tissue retrieval forceps and removed them from the abdominal cavity. All ovariectomies were successful, and the EnSeal® device provided adequate haemostasis in all cases. Although ligatures made with surgical suturing material and surgical clamps also enable effective haemostasis, their adequate positioning requires practice and is more time consuming than tissue sealing.

In the case of five canine patients, we removed a unilateral intra-abdominal cryptorchid testis by a laparoscopic method. These patients were also positioned in dorsal recumbency, in Trendelenburg position. The optical portal was created a few centimetres caudal to the umbilicus while the working channels at the midpoint of the distance between the umbilicus and the pubic bone, slightly lateral to the rectus abdominis muscle. The cryptorchid testis and the epididymis were fixed with a manipulator and, after terminating their blood supply and separating them from the associated soft tissues with the EnSeal® device, they were removed from the abdominal cavity through the abdominal wall wound within the working channel with the manipulator. All interventions were successful and the EnSeal® device provided adequate haemostasis in all cases.

In four dogs with a non-ruptured splenic tumour we performed a (palliative) total splenectomy to increase the expected time of survival. The intervention was performed via median laparotomy. The affected spleen was lifted out through the abdominal wound and then the branches of the *arteriae et venae lienales* as well as the *arteria et vena gastroepiploica sinistra* and the *arteriae et venae gastricae breves* were successively sealed with the EnSeal® device at the hilum of the spleen. The other method of removal was to open the mesenteric sac and to find and seal the splenic artery (*arteria lienalis*) distal to the exit point of the branches supplying the left lobe of the pancreas. The EnSeal® device proved to be simple to use, safe and efficient, it effectively sealed the treated blood vessels during all activations and shortened the operative time. To the best of our knowledge, splenectomy performed by the use of the EnSeal® device has not been reported in the literature so far.

In two canine patients we used the EnSeal[®] device for performing mesenteric skeletisation prior to entero-resection and enteroanastomosis. In one of these two dogs, a foreign body stuck in the small intestine caused complete occlusion and severe circulatory disturbance. The other dog was subjected to surgery because of an adenocarcinoma in the wall of the small intestine. Exploration of the abdominal cavity was done by median laparotomy in both cases. We lifted out the affected intestinal segment and then marked the intestinal segment to be removed and the sealing points of the arcade-like jejunal blood vessels supplying it. With the EnSeal[®] device we sealed the blood vessels, removed the designated intestinal segment and then united the intact ends. To the best of our knowledge, mesenteric skeletisation performed by the use of the EnSeal[®] device in dogs has not been described in the literature so far.

In one patient, we removed the caudal pole of the right pancreatic lobe via upper median laparotomy because of an early-stage insulinoma. We lifted the proximal duodenum and then inspected and non-traumatically palpated the pancreas. We identified the tumour and removed it with the EnSeal[®] device without causing any damage to the exocrine function. Finally we closed the opening created on the mesoduodenum. To the best of our knowledge, complete or partial pancreatectomy performed by the use of the EnSeal[®] device has not been described in the literature so far.

In four patients, we carried out laparoscopic liver biopsy using the EnSeal[®] device in order to characterise the existing hepatitis. The dogs were positioned in dorsal recumbency, and the optical portal was established a few centimetres caudal to the umbilicus. The working channels were created at the midpoint of the distance between the umbilicus and the pubic bone, slightly lateral to the rectus abdominis muscle, in a paramedian plane. Because of the diffuse nature of the hepatic lesion, the site of biopsy sampling was not predetermined. With the EnSeal[®] device we obtained V-shaped samples from the marginal parts of the hepatic lobes and lifted them out of the abdominal cavity through the portal wound. As compared to the percutaneous technique, laparoscopic liver biopsy offers the benefits of good visualisation, accurate positioning and the lack of need for ultrasound guidance, while its advantages over laparotomic techniques include minimal invasiveness and low time requirement. To the best of our knowledge, there is no report in the literature that describes liver tissue resection or liver biopsy performed by the use of the EnSeal[®] device in dogs.

In one dog, we carried out thoracoscopic lung biopsy with the EnSeal[®] device for histological examination. The patient was positioned in lateral recumbency and, after making skin

incisions and blunt dissection of the intercostal muscles, we established the thoracoscopic portals in the right 3rd, 5th and 9th intercostal spaces. Because of the diffuse nature of the lung lesion the site of biopsy sampling was not predetermined in this case either. With the help of the manipulator and the EnSeal[®] device, we took V-shaped biopsy samples from the marginal parts of the lung lobes and removed them through the portal wound. The EnSeal[®] device proved to be easy to use and safe. It provided proper haemostasis and prevented air leakage in the sealed lung areas. As compared to the percutaneous fine-needle aspiration technique, thoracoscopic biopsy enables direct visualisation, does not require ultrasound or fluoroscopic guidance and makes it possible to take larger biopsy samples, while its benefits over thoracotomy procedures are minimal invasiveness and short operative time. To the best of our knowledge, no paper describing lung biopsy performed by the use of the EnSeal[®] device has been published in the literature so far.

In two dogs with idiopathic intrapericardial effusion we performed thoracoscopic partial pericardiectomy using the EnSeal[®] device. We positioned the patients in lateral recumbency and created the portals in the right 4th, 6th and 8th intercostal spaces. Avoiding the phrenic nerve, we partially removed the intrapericardial fluid content, then with the help of a manipulator and the EnSeal[®] device we created a window on the pericardium. We lifted out the removed pericardial tissue through the portal wound and then drained the thoracic cavity. To the best of our knowledge, complete or partial pericardiectomy performed by the use of the EnSeal[®] device has not been reported in the literature so far.

In three dogs we carried out unilateral eye enucleation because of atrophy, an intraocular tumour and glaucoma unresponsive to therapy, respectively. For the removal of the affected eyeball, the patients were positioned in sternal recumbency on a vacuum mattress, with their heads slightly lifted and turned to the side. After lateral canthotomy, we separated the conjunctiva from the eyeball concentrically along the limbus, then separated the conjunctiva, Tenon's fascia and the extraocular muscles from the sclera. Avoiding laceration of the eyeball, with the EnSeal[®] device we sealed the optic nerve and the blood vessels in one step. We excised the third eyelid and its gland, then removed the edges of the lower and upper eyelids, sparing the angular vein (*vena angularis oculi*) in the medial canthus of the eye. Finally, we unified the conjunctiva, the septum, Tenon's fascia and the eyelids with sutures. To the best of our knowledge, eye enucleation performed with the help of the EnSeal[®] device has not been published in the literature so far.

The result of blood vessel sealing and dissection can, and should, be evaluated immediately during surgery (presence or absence of bleeding), while the histological examinations

provide subsequent information about the histomorphological changes (occlusion of the lumen of the treated blood vessels). The blood vessels and tissue samples sealed and removed with the EnSeal[®] device were preserved in 8% buffered formaldehyde solution. After preparation and processing, the sections stained with haematoxylin and eosin were examined in order to evaluate the histomorphological parameters of the lesions. In all cases, histological examination demonstrated a gradual separation of the intact and the treated, compacted, dehydrated and homogenised tissue areas as well as occlusion of the lumen of the blood vessels treated with the tissue-sealing device.

Both the conventional and the minimally invasive interventions passed off as planned. When inducing pneumoperitoneum during the laparoscopic interventions, iatrogenic mechanical injury did not occur in any of the cases. The EnSeal[®] device worked effectively and safely for all types of surgery examined (ovariectomy, cryptorchidectomy, complete splenectomy, mesenteric skeletisation, partial pancreatectomy, liver biopsy, lung biopsy, partial pericardiectomy, eye enucleation) and in all cases of intervention (N=62). It proved to be suitable for grabbing the desired blood vessels and tissue bundles, for occluding the lumen of the blood vessels treated and for dissection of the tissue bundles. The duration of blood vessel and tissue bundle sealing greatly depended on the diameter of the structures to be sealed, but the mean duration did not exceed the time required for making ligatures. Owing to the built-in cutting blade, the sealed tissue was cut in all cases. In order to prevent thermal injuries, the EnSeal[®] device was activated only if the jaws of the device, the target organ and its surroundings were equally well visible, and when the jaws of the device were in contact with the target organ only. After activation, we provided sufficient time to allow the temperature of the device to decrease. Notable thermal injury did not occur in the surrounding tissues. The amount of generated surgical smoke was minimal. The ovaries, the cryptorchid testes, the biopsy samples and the pericardial fragments could be removed through the gently dilated abdominal wall wound within the portal. The net operative times were not longer than those required by conventional techniques, and they tended to decrease towards the end of the series of operations. The minimally invasive techniques reduced the patients' postoperative discomfort and the time of their recovery, and they provided aesthetically more acceptable results. Like the results of both the preliminary trials on pigs and the surgical interventions on horses, our intraoperative observations and histological examinations made in connection with soft-tissue operations in dogs unanimously demonstrated the safe and effective operation of EnSeal[®]. On the basis of our results, we recommend the optional use of the EnSeal[®] device for surgical interventions of the above types.

5. NEW SCIENTIFIC RESULTS

- We studied the burst pressure of blood vessel segments sealed with the EnSeal[®] device under standardised conditions. The burst pressure of the vessel segments tested was 873.89 ± 120.57 (425–1555) mmHg, and it was multiple times higher than the physiological blood pressure value in all cases.
- By the use of the EnSeal[®] device, we successfully sealed also blood vessels falling outside the specifications of the device (having a diameter of 8–12 mm), and established that the sealed vessels had suprphysiological (>360 mmHg) burst pressure.
- We determined the maximum burst pressure resistance values of sealed vessels complying with the specifications of the device (blood vessels 2–7 mm in diameter). The highest value (1555 mmHg) was measured on a carotid artery sample 5 mm in diameter.
- On the basis of thermocamera recordings we studied the temperature profiles of the activated handpiece (perpendicular to the longitudinal axis). The profile can be characterised by a bell-shaped curve and a W-shaped thermal shadow at the peak of the former. The temperature peaks occur at the edges of the jaws and along the longitudinal axis.
- On the basis of our thermocamera recordings, we determined the maximum surface temperature of the surrounding tissues during activation of the handpiece, and found it to be 69.25 ± 0.98 °C.
- On the basis of our thermocamera recordings, we determined the maximum surface temperature of the treated tissues at the end of handpiece activation, and found it to be 66.89 ± 0.66 °C.
- On the basis of our thermocamera recordings, we determined the rate of decrease of maximum surface temperature values after the activation of the handpiece. The average temperature decrease of the tissues was 14.50 ± 2.97 °C after the first 4 seconds and further 3.90 ± 0.42 °C after 8 seconds. After 10 seconds, the temperature fell below 40 °C in all cases. The temperature of the examined tissues in the period from one second before the end of activation to the 10th second after the end of activation can be described with the following polynomial equation: $y(^{\circ}\text{C}) = -0.0139*(x_{(s)}+2)^4 + 0.3284*(x_{(s)}+2)^3 - 2.3627*(x_{(s)}+2)^2 + 2.6372*(x_{(s)}+2) + 68.661$.

- As the degree of thermal injury is closely correlated with the maximum temperature but it also depends on the duration of treatment, the interval between two treatments, the perfusion and other biothermomechanical properties of the tissue treated, we used histological methods for evaluating the thermal injury. We successfully used the NBTC enzyme histochemical procedure, found it to have outstanding efficacy and recommend it for use as a standard for histological comparisons of the thermal injury caused by different performance levels and application times of different electromagnetic energy-based devices and for quantitative determination of the dimensions of the microscopic thermal injury zone.
- Using the NBTC enzyme histochemical procedure, we studied the effect of temperature on the enzyme activities of the tissues. The enzyme proteins responsible for producing a colour reaction maintained their activity at both $-20\text{ }^{\circ}\text{C}$ and $-80\text{ }^{\circ}\text{C}$ during freezing but they became irreversibly inactivated at $64\text{ }^{\circ}\text{C}$ during heating.
- We determined and statistically evaluated the total and the collateral microscopic thermal injury zone in different tissues, under standardised conditions. In our samples, the average MTZ_t was $5.55\pm 0.08\text{ mm}$ and the average MTZ_{lat} was $0.28\pm 0.04\text{ mm}$, and the differences between the values obtained for the tissue types examined were not significant.
- We started the application of the EnSeal[®] vessel- and tissue-sealing system in the Hungarian veterinary clinical practice, and performed novel-type surgical interventions with the help of this device. In several cases, we combined the tissue sealing with minimally invasive operative techniques.
- We reported laparoscopic cryptorchidectomy and ovariectomy performed by the use of the EnSeal[®] device on sedated standing horses for the first time in the literature.
- On dogs, we successfully carried out further novel-type surgical interventions hitherto not reported in the literature, by the use of the EnSeal[®] device, such as splenectomy, mesenteric skeletisation, partial pancreatectomy, liver biopsy, lung biopsy, partial pericardiectomy and eye enucleation. We are continuously expanding the fields of use of the device.

6. PUBLICATIONS REPORTING THE RESULTS OF THE POSTGRADUATE RESEARCH

Papers written on the subject of the postgraduate research, published/accepted for publication in peer-reviewed scientific journals having an Impact Factor

- Dunay, M., Németh, T., Bodó, G. (2008): Az elektrosebészet alapjai [The bases of electrosurgery]. Magyar Állatorvosok Lapja 130 (8) 498-504, IF: 0,088
- Dunay, M., Jakab, Cs., Németh, T. (2011): Evaluation of EnSeal[®], an adaptive bipolar electrosurgical tissue-sealing device. Acta Veterinaria Hungarica, accepted for publication on October 11, 2011. IF: 1,264
- Dunay, M., Németh, T., Makra, Z., Izing, S., Bodó, G. (2011): Laparoscopic cryptorchidectomy and ovariectomy in standing horses using the EnSeal[®] tissue-sealing device. Acta Veterinaria Hungarica, accepted for publication on October 11, 2011. IF: 1,264

Conference presentations and conference abstracts connected with the subject of the postgraduate research and published in technical journals

- Dunay, M. (2006): Új elektrosebészeti technikák vizsgálata [Study of new electrosurgical techniques]. Magyar Tudományos Akadémia Állatorvos-tudományi Bizottsága, Akadémiai beszámoló, Klinikumok és gyógyszerstan [Committee on Veterinary Science, Hungarian Academy of Sciences, Academic Reports, Clinical Sciences and Pharmacology].
- Izing, S., Bodó, G., Dunay, M., Fürst, A. E. (2006): Hasúri rejtett here és petefészek eltávolítása álló lovon [Removal of intra-abdominal cryptorchid testis and ovary on standing horses]. MÁOK (Magyar Állatorvosi Kamara) és a Hungarovet éves országos konferenciája, Budapest [Annual National Conference of the Hungarian Veterinary Chamber and Hungarovet, Budapest].
- Izing, S., Bodó, G., Dunay, M., Fürst, A. E. (2006): Hasúri rejtett here és petefészek eltávolítása álló helyzetű lovon [Removal of intra-abdominal cryptorchid testis and ovary on standing horses]. XIV. Lógyógyászati Kongresszus, Üllő [XIVth Congress of Equine Medicine, Üllő].

- Dunay, M., Bodó, G., Izing, S. (2007): Endoszkópos beavatkozások álló lovon szövethégesztő rendszer segítségével [Endoscopic interventions on standing horses with the help of a tissue-sealing system]. MTA Állatorvos-tudományi Bizottsága, Akadémiai beszámoló, Klinikumok és gyógyszerstan [Committee on Veterinary Science, Hungarian Academy of Sciences, Academic Reports, Clinical Sciences and Pharmacology].
- Bába, A., Bodó, G., Kulik, M., Izing, S., Dunay, M. (2007): Petefészek-daganat lóban – Új műtéti megoldás első hazai esete [Ovarian tumour in a horse – First description of a new operative solution in Hungary]. XIV. Szaporodásbiológiai Kongresszus [XIVth Congress of Reproduction].
- Dunay, M. (2007): Elektrosebészeti eszközök alkalmazása a lágyszervi sebészetben [Use of electro-surgical instruments in soft-tissue surgery]. HSAVA (Magyar Kisállatgyógyász Állatorvosok Egyesülete) Országos Konferenciája [National Conference of the Hungarian Small Animal Veterinary Association].
- Dunay, M., Tóth, A., Jakab, Cs., Németh, T. (2009): Elektrosebészeti beavatkozások kollaterális hőkárosító hatásának vizsgálata [Study of collateral thermal injury caused by electro-surgical interventions]. MTA Állatorvos-tudományi Bizottsága, Akadémiai beszámoló, Klinikumok és gyógyszerstan. [Committee on Veterinary Science, Hungarian Academy of Sciences, Academic Reports, Clinical Sciences and Pharmacology].
- Dunay, M., Tóth, A., Németh, T. (2009): Experimental assessment of collateral thermal injury caused by the EnSeal[®] electro-surgical tissue sealing system. Poster. 18th ECVS (European College of Veterinary Surgeons) Annual Scientific Meeting, Nantes, France.
- Dunay, M., Tóth, A., Németh, T. (2009): Experimental assessment of collateral thermal injury caused by electro-surgical tissue-sealing. Conference Abstract. Veterinary Surgery, 2009. 38, E9

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