ULTRASONIC VS. RADIOFREQUENCY ENERGY SEALING OF THE CYSTIC DUCT IN CHRONIC CHOLECYSTITIS PATIENTS

by

KIMBERLY E. MARTIN

B.A., University of Missouri-Columbia, 1996

MSCS, University of Colorado Anschutz Medical Campus, 2013

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This thesis for the Doctor of Philosophy degree by

Kimberly E. Martin

has been approved for the

Clinical Science Program

by

Heather Haugen, Chair
John E. Hokanson, Advisor
Thomas N. Robinson
Paul Montero
Anna E. Barón

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Martin, Kimberly E. (Ph.D., Clinical Science)
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ABSTRACT

Laparoscopic Cholecystectomy is one of the most common abdominal operations performed in the United States today. Currently, surgical clips are the gold standard to close the cystic duct during the operation, but 0.5-3.3% of patients experience a cystic duct leak. Experimental use of commercially available Energy-Based Vascular Sealing Devices (EBD) to close the cystic duct has been described in the surgical literature. There are potential advantages to using an EBD including reduction in bile leak, reduced instrument exchange, reduced post-operative pain, and reduced operating time. To date there is little evidence to support the use of a vascular EBD in the cystic duct, and there is no EBD optimized specifically for this structure. The collagen to elastin ratio (C/E) of the vascular tissue has been shown to affect seal quality in animal and human cadaver vasculature models, and may also have a similar effect in other tubular structures such as cystic duct, however this has not been directly examined. Our objective is to determine if energy-based devices adequately seal human cystic duct, and if the energy source impacts seal quality given collagen to elastin ratio and cystic duct diameter.

The form and content of this abstract are approved. I recommend its publication.

Approved: John E. Hokanson
“In questions of science, the authority of a thousand is not worth the humble reasoning of a single individual.” — Galileo Galilei

I am the first person in my entire family to go to college. I am a firm believer that none of us achieves anything completely on our own. I am humbled and grateful for all the help through the years. I owe a special debt of gratitude to these individuals because without them there would be no PhD:

My wife Camille, who is my partner in all things and a shining example of the kind of person I hope to be someday.

Dr. Tom Robinson, whose dedication to evidence-based medicine, to patients, and to students is selfless and unwavering.

The patients who participated in the trial, who allowed access to their most private and personal information in the name of science, on the small chance that we can help future patients by changing the status quo for testing these devices.
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CHAPTER I
INTRODUCTION

What drugs will not cure, the knife will; what the knife will not cure, the cautery will; what the cautery will not cure must be considered incurable.

–Hippocrates

Cholelithiasis or gallstones in the biliary tract is highly prevalent around the world [1-6]. Gallstones result in cholecystitis about 20% of the time however, given the prevalence of cholelithiasis, this translates into one of the most common indications for abdominal surgery [7]. The etiology of cholelithiasis is multifactorial, and like many conditions, propensity for the development of gallstones results from a combination of environmental and lifestyle factors, and genetic disposition [1]. In general, gallstones are more common in the Americas, Europe and Asia than in Africa, with variation due to sex and ethnicity within geographic regions [5]. The populations with the highest prevalence are southwestern Native Americans such as the Pima Indians, and indigenous Mexican and South American Indians, in which up to 70% of the population may have gallstones [5]. Individuals with high body mass index, age over 40 years, rapid weight loss, female sex, multiparity and inadequate cardiovascular exercise are at higher risk for having gallstones [5].

Clinical gap

Due to the pervasiveness of cholelithiasis (gallstones) and accompanying cholecystitis (inflamed gallbladder and ducts) across the world, laparoscopic cholecystectomy (LC) is one of the most common abdominal operations performed today [7]. The laparoscopic approach has essentially replaced open cholecystectomy and is the gold standard treatment for cholecystitis [7, 8]. Bile leaks related to migration or
mechanical failure of surgical clips used to close the cystic duct (CD) are almost non-existent during the open procedure; however, bile leak due to clip failure occurs in 0.5-3.3% of patients treated laparoscopically [9-13]. Bile leak may result in serious and life-threatening complications such as bile peritonitis, fistula or biloma [14]. Given that there are 750,000 to 1 million LCs performed per year in the United States alone, this translates into between 1,750 and 33,000 and patients who experience this serious complication each year, with substantial healthcare economic impact [7, 13].

Energy-based devices (EBDs) are increasingly used in laparoscopic operations in place of traditional mechanical methods to seal other tissues in addition to vasculature. Both ultrasonic (US) and radiofrequency bipolar (BP) EBDs have been used experimentally to seal the cystic duct in an effort to reduce pain, speed up the operation, reduce instrument exchange, and potentially reduce complication rates related to clip migration and capacitive coupling from monopolar instruments to the clip. However, current EBDs are not optimized for sealing the cystic duct, there is no validated test model and outcomes are variable [15-19].

**Evidence gap**

To date, there is little basic science data in human tissue to allow for optimization of the devices for the cystic duct or to determine which energy source is most effective. Adequate collagen content is critical to achieving a robust seal in vessels, and it is a reasonable hypothesis that this is true of other tubular biological structures such as the cystic duct [20-24]. The metric of burst pressure is used as a surrogate for the physiologic pressure that a biological structure can withstand i.e. the systolic blood pressure for vascular sealing, and bile pressure for cystic duct sealing. Currently there are no data published on the collagen and elastin content of the cystic duct or the range of diameters observed across disease states, and there are very little pressure data on human tissue BP cystic duct seals. Although one recent scanning electron microscopic study does
document burst pressure and observed collagen denaturation in eight excised human sealed cystic ducts, the results of the collagen content are based on histological appearance, and thus are qualitative [25]. A few other studies have documented the burst pressure of porcine common bile ducts, porcine cystic ducts, porcine common bile ducts and human cystic ducts but the test methods, and results are variable [18, 19, 26, 27]. There is variability in test methodology for vascular testing as well. The inconsistent methodology documented in the literature for burst pressure testing in vasculature has been critically reviewed in a prior publication by our group with a call for standardization, and many of the same principles may be applied to cystic duct testing [20] (Appendix F).

Industry standard testing for FDA market clearance for vascular sealing is currently performed by a bench burst pressure test on excised healthy porcine renal arteries [29]. This test simulates the maximum amount of pressure a seal can withstand, and is the bench model for the physiologic blood pressure exerted on seals in vivo in surgery prior to clot formation and healing (Appendices C-F) [21-23]. No evidence exists validating that healthy porcine renal arteries are the most clinically relevant model i.e. similar in terms of collagen content or burst pressure. Recent data suggest that porcine renal arteries are not an appropriate clinical vascular model, and this may contribute to clinical seal failures [21-23]. Given the similarity of the cystic duct to vasculature in size and shape, some investigators have adopted the porcine cystic duct burst pressure as a model for human cystic ducts despite a similar lack of evidence for similarity in the collagen to elastin ratio (C/E) and the suitability of the porcine model [19, 29, 30]. This study will be the first step toward the development of an appropriate model for clinical use in diseased human cystic ducts.

Further study is needed in human tissue to investigate associations between C/E, cholecystitis severity and burst pressure. A better understanding of the basic relationship between tissue state and type in terms of C/E, suitability of the porcine
model, viscoelasticity and seal patency may allow for improvement of the technology leading to a reduction in the currently observed rate of bile duct leak. Furthermore, this translational research may facilitate the introduction of the technology into alternative tissues such as lung or liver parenchyma sealing [31-38]. By gathering data on the relationship of burst pressure to C/E and diameter in the cystic duct as well as comparing the effectiveness of different energy sources, this study will establish which types of energy are safe and effective for sealing the cystic duct, and will lay the groundwork for the optimization of EBDs for use in sealing cystic ducts.

As a preliminary step to the larger investigation, we have been the first group to collect data on the basic morphological components (C/E) of sealed vasculature in order to better understand the quantitative effect of tissue characteristics and the first to propose a robust and repeatable test methodology. It is important to apply these principles to every tissue type sealed including the cystic duct (Appendices C-F).

**Significance**

This work will help surgeons to make evidence-driven decisions about the use of EBDs, and will provide data to explore the relationship between collagen and elastin content and seal quality in human cystic ducts. The potential for optimization or the specific design of EBDs for cystic duct sealing and a reduction in the rate of bile leak as well as translation into sealing other tissues exists through a better understanding of the basic mechanism of sealing tissues [11-20]. An energy-based device optimized for the cystic duct would fundamentally change the most common laparoscopic operation performed by general surgeons. Data confirming the relationship of C/E to burst pressure in the cystic duct as previously shown in blood vessels would challenge the current criteria used by FDA to approve energy-based devices for the market.

In addition to the sheer volume of LCs performed each year, the cystic duct is an impactful target due to the severity of the complications associated with bile leak such as
bile peritonitis, fistula and biloma. Currently the rate of bile leak rate with standard clips has been documented from 0.5-3.3% in laparoscopic cholecystectomy [39, 40]. An EBD may reduce the rate of bile leak compared to standard surgical clips, however the type of EBD best suited to sealing the cystic duct is unknown. Both US and BP EBDs melt the collagen and elastin in the tissue being sealed, but there are fundamental differences in the way the two device types generate heat and in the feedback mechanisms used to prevent and reduce thermal spread.

A number of investigators have suggested that using an US EBD for LC reduces instrument exchange, speeds up the operation, reduces length of stay and post-operative pain, and may allow for a reduction in the number of ports required [9, 26, 27, 41-43]. However, very little data exists for the BP device. Given that the BP device is FDA cleared to seal larger vessels (7 mm) than the US device (5 mm), BP energy may be a better energy source for sealing larger (> 5 mm) inflamed cystic ducts. Other investigators have experimented with clinical (leaving a sealed duct in vivo) cystic duct sealing. However, results are variable for the outcome of post-operative leak of cystic ducts sealed with either BP or US energy [9, 26, 27, 41-43]. Optimization of an EBD for cystic duct sealing in LC has the potential to reduce bile leak rates and the associated human suffering and health economic impact of bile leak-related complications such as biliary peritonitis, which require extended hospital stays and intensive clinical care.

Objectives

Research question: Do energy-based surgical devices adequately seal human cystic duct, and does the energy source impact seal quality given duct diameter and collagen and elastin content?

We took an iterative approach to this question. First we conducted a pilot animal study to determine burst pressures of sealed porcine cystic ducts. Next, to determine
feasibility for healing, we conducted a survival animal study in a porcine model in which 10 pigs underwent laparoscopic cholecystectomy utilizing BP energy to seal the cystic duct. One half of the seal was burst immediately, and after seven days the animals were sacrificed and the other half of the seal on the cystic duct stump was excised and burst tested. After that, we conducted a prospective cohort study to evaluate the feasibility of sealing the cystic duct with BP energy in patients with chronic cholecystitis, and to gain a better understanding of the fundamental morphology of the human cystic duct. Finally we conducted a randomized controlled trial to compare burst pressures after US or BP energy seals in excised human cystic ducts.

**Porcine study objectives**

1. Determine if burst pressures were at an acceptable level in porcine cystic ducts.
2. Determine incidence of bile leak in a survival porcine model after sealing the cystic duct with BP energy.
3. Determine the burst pressure of cystic ducts sealed with BP energy after seven days of healing and compare it to the other half of the seal burst immediately after excision.

**Cohort study objectives**

1. Determine the distribution of human cystic duct burst pressures sealed with BP energy.
2. Quantify the range of human cystic duct diameters and wall thicknesses.
3. Understand if the predictors of vascular burst pressure are also associated human cystic duct burst pressure.

Once this single arm study was complete, we conducted a randomized controlled trial in order to understand which energy source performed better in the cystic duct.
Randomized controlled trial objectives

1. Compare the mean burst pressure of human cystic ducts sealed with US energy or BP energy.
2. Evaluate the relationship between collagen and elastin content, human cystic duct diameter and burst pressure.

History of energy-based devices

Since earliest recorded history, maintaining hemostasis has been recognized as a critical and fundamental aspect of successful surgery. The application of heat in a surgical setting dates back to as early as 3000 BC, but conductive methods of heat transfer can result in extensive collateral tissue damage due to the uncontrolled nature of the tissue and energy interaction. A brief synopsis of the evolution of energy-based surgical devices (EBDs) follows.

Monopolar electrosurgery

In the beginning of the 20th Century, alternating electrical current found a number of applications in surgery due in large part to the work of French scientist Jacques-Arsène d’Arsonval. His research demonstrated that alternating current could be passed through tissue without producing any physiologic effects outside of heating, and that if the frequency of alternating current was high enough, the unwanted side effect of muscle stimulation and contraction did not occur [45].

Collaboration between Dr. William Bovie and Dr. Harvey Cushing introduced high frequency monopolar electrosurgery to clinical use in the 1920s. The original Bovie generator delivered alternating current of very high frequency that created heat by ionic agitation (kinetic energy) at the tissue–electric interface. The heat affects proteins in vascular walls and plasma, creating a coagulum. Early monopolar devices did not
provide for precise control of energy delivered to tissues, and unwanted collateral tissue damage including carbonization was a concern.

**Introduction to bipolar radiofrequency electrosurgery**

In the 1940s, bipolar electrosurgical instruments were developed. Bipolar instruments allowed surgeons to direct the application of electrosurgical energy to specific tissue held between the electrodes of the instrument, usually in the form of forceps. Bipolar electrosurgery offered potential benefits over monopolar: No return electrode needed to be attached to the patient; effective at lower energy levels, minimizing collateral tissue damage; coagulation in wet fields; compatible for patients with pacemakers and implantable defibrillators [46].

**Bipolar electrosurgery: Closed-loop control, first improvement**

Continuous microprocessor feedback in a “closed loop control system” was introduced in the 1990s [47]. Closed-loop control systems sense changes in tissue impedance and adjust the thermoelectric output accordingly. Energy output increased in response to increased tissue independence (Ohms law), and real-time power adjustments allowed lower output settings to be used, limiting collateral damage and allowing for the ligation of larger vessels. The introduction of a foreign body (suture, clips, etc.) was known to increase the patient’s potential for infection, and the possibility of using closed-loop control to create an autologous clip composed of the patients own proteins for vascular ligation was tremendously compelling.

**Bipolar electrosurgery: Vascular ligation, present state**

In 1996, Kennedy et al. were the first to study the use of closed-loop bipolar electrosurgery for vascular ligation. Working with the variables of jaw pressure, electrical current, bipolar jaw pressure and energy delivery time, this group developed and
subsequently optimized a bipolar energy system that was able to reliably seal vascular structures. They validated the system by measuring acute vascular burst pressure. The generator they developed sensed the characteristics of the tissue being sealed and provided a customized output algorithm for optimized energy delivery. Once the endpoint of the energy delivery algorithm was reached, the generator automatically ceased delivering energy and the user was alerted of this via an audio signal. A more in-depth discussion of the mechanism of action of these devices follows.

**Ultrasonic surgery**

Like radiofrequency energy, ultrasonic energy (US) has been broadly utilized for hemostasis and tissue ablation in medicine for more than 50 years. In 1927, Wood and Loomis were the first to investigate the tissue effects of high intensity ultrasonic energy [45]. Using animal models such as fish, mice and frogs they discovered that frequencies of 100-700 KHz resulted in intra-abdominal bleeding in addition to the intended ablation at the surgical site. As a result, they reduced the vibrational intensity and discovered that a more localized effect could be achieved. This study has been described as the first safety and efficacy study of the piezoelectric effect on tissue. The first human use was described in the 1950s in dentistry for removal of calculus from teeth. Since then, broad use of ultrasonic energy for cavitation, ablation and cutting of soft tissue, bone, vascular plaques and all manner of tissue has been described [48].

**The Cavitron ultrasonic surgical aspiration system**

The Cavitron ultrasonic surgical aspiration system (CUSA) was introduced in the 1970s. This device is sometimes referred to as an “ultrasonic scalpel.” The CUSA fragments and aspirates tissues with mechanical energy delivered by direct contact with the tip of a transducer vibrating at 23,000 KHz. Softer tissue such as liver parenchyma or brain tissue may be readily ablated and dissected from more robust tissues such as
nerves and blood vessels. Small vessels may be cauterized in a manner similar to
monopolar electrosurgery. However, as we now know, larger vessels require a device
with jaws that can clamp with precise pressure.

**Ultrasonic coagulating shears and vascular ligation**

Ultrasonic coagulating shears were introduced in the 1990s following the growing
acceptance for laparoscopic surgery. Unlike the CUSA, which is an aspirator, these
devices had jaws which were more similar to the bipolar radiofrequency devices in
appearance and function. Beginning in 1995, a number of authors investigated the use of
US for vascular sealing in a wide array of surgical procedures. Swanstrom and Pennings
described the first study comparing US to vascular clips for control of small gastric
vessels. Other authors rapidly followed with publications describing the use of the device
for vascular hemostasis and dissection in laparoscopic hysterectomy, Nissan
fundoplication, laparoscopic cholecystectomy and a plethora of other surgical
procedures. Some authors compared US to monopolar hook cautery for dissection,
hypothesizing that the US surgery would result in reduced smoke and an improved
safety profile by lowering the risk of capacitive coupling to other instruments. A more in-
depth description of the mechanism of types of EBD follows in the next section.

**Mechanism of action of energy-based devices**

EBDs operate by generating heat via either ultrasonic (US) or bipolar
radiofrequency energy (BP), and both types of EBDs have been used for decades to
achieve intraoperative hemostasis without the introduction of a foreign body [20, 49]. In
the simplest terms, these devices use a combination of heat and precise pressure to melt
the major proteins (collagen and elastin) in the vascular wall [20, 49]. This amalgam is
then allowed to cool under pressure and forms an “autologous clip” (Figure 1) [20]. The
earliest example of this method is current applied via a monopolar “bovie” device to
standard surgical steel hemostats while clamping a vessel [20, 49]. Coagulation and vessel seal patency were determined by the surgeon — a technique that only worked on relatively small diameter vessels and caused extensive collateral damage to adjacent structures via thermal spread [20, 49].

**Figure 1. Energy-based device seal.** (a) Bipolar device sealing artery; (b) resulting bipolar seal; (c) porcine carotid artery stained with Masson’s trichrome. Collagen is blue, denatured collagen is dark purple, elastin is black, smooth muscle is red.

**Ultrasonic mechanism**

Ultrasonic energy devices operate via mechanical, kinetic energy. Electrical energy is converted into high frequency vibration of 55,000 kHz with a piezoelectric transducer in the hand piece. The vibration of the ferroelectric ceramic crystals in the transducer is subsequently translated into ultrasound waves causing the jaws of the device to vibrate longitudinally. The mechanical vibration of the jaws of the device against the tissue produces heat via friction. A heat-based software algorithm automatically adjusts the frequency of vibration to cool the jaws and to avoid charring of the tissue [50]. The specific FDA clearance language is listed below:

“Indications for use include soft tissue incisions when bleeding control and minimal thermal injury are desired. The device can be used as an adjunct to or substitute for electrosurgery, lasers, and steel scalpels in general, plastic, pediatric, gynecologic, urologic, exposure to orthopedic structures (such as spine and joint space) and other open and endoscopic procedures. The Device can be used to coagulate isolated vessels up to 5 mm in diameter.”
Bipolar mechanism

Bipolar energy-based devices operate via radiofrequency electrical energy. The jaws of the BP device have two electrodes incorporated into them, and when closed on tissue, complete a circuit. Heat is generated due to the impedance of the tissue as the alternating current passes through it between the jaws of the device. The frequency of the energy must be in excess of 300,000 kHz in order to avoid cardiac stimulation or neuro-stimulation of skeletal muscle. An impedance-based algorithm automatically adjusts the power output as sealing progresses (impedance rises as the tissue is cooked and dried) to prevent charring of the tissue [20, 49]. The specific FDA clearance language is listed below:

“Indications for use include general open and minimally invasive procedures including urologic, vascular, thoracic and thoracoscopic, and gynecologic procedures where ligation and division of the vessels is performed. These procedures include: laparoscopically assisted vaginal hysterectomy, Nissen fundoplication, colectomy, adhesiolysis, oophorectomy, etc. The device has not been shown to be effective for tubal sterilization or tubal coagulation for sterilization procedures, and should not be used for these procedures. One-step sealing devices can be used on vessels and lymphatics up to and including 7 mm. and tissue bundles.”

Seal quality evaluation: Burst pressure testing

Seal reliability has historically been evaluated throughout the device development process via the metric of “burst pressure” using fresh excised renal arteries or cystic ducts from young, healthy animals as the testing model, and this method has become the Food and Drug Administration and industry standard [21-23, 28]. Briefly, the burst pressure is measured by inserting a cannula into the open end of a sealed, excised vessel. The cannula is connected via tubing to a pressure transducer and a pump, which fills the vessel at a fixed rate until the seal bursts (Figure 2). The pressure at burst is automatically recorded with the pressure transducer and is referred to as the “maximum burst pressure.”
Factors influencing burst pressure and potential confounders

There is an inverse relationship between the diameter of a tubular structure and maximum burst pressure due in part to Laplace’s law (Wall Tension = Radius x Pressure). This necessitates that all experiments control for diameter in assessing contributors to burst pressure. A limitation of the literature is that despite evidence that other variables may influence burst pressure, only vessel diameter is routinely controlled for in device comparative effectiveness studies or in safety and efficacy experiments intended for FDA clearance of new devices for vessel sealing [20]. In the current cystic duct literature, even diameter is not measured or controlled for. A major factor that is overlooked in the literature describing burst pressure testing, with the exception of our previous publications on the topic, is the inherent composition of the structures themselves. Collagen and elastin are the major components in a vascular seal, and the ratio of collagen to elastin (C/E) has been shown to vary in physiologically discrete vessels. In a canine model, relative to mesenteric arteries and femoral arteries, renal arteries have approximately 50% more collagen [51]. In a porcine model, renal arteries have about 50% more collagen than femoral arteries and burst at significantly higher pressures [21]. It is currently unknown whether human cystic duct corresponds to the current testing model of porcine cystic ducts in terms of collagen content and/or burst pressure.

Collagen content affects not only the amount of robust material incorporated into the seal i.e. a critical amount of collagen is required for a robust seal, but is also a critical
determinant of the distensibility or the overall elasticity of the structure sealed [52]. Collagen and elastin each have different Young’s modulus of elasticity values, with collagen the more rigid, “tough” protein and elastin the more elastic and flexible protein. The ratio of these two proteins in the vessel or duct wall and their interaction play a key role in determining the overall pliancy of the vessel or duct and the wall tension when the vessel or duct is distended [52]. Given the mechanism of burst pressure testing, which models the pressure exerted on an arterial or cystic duct seal left \textit{in vivo}, one could surmise that distensibility or how much the vessel or duct can stretch and expand like a balloon under pressure (Figure 2c) could have a major impact on the tension in the vessel or duct wall and on the force exerted on the sealed end. This would lead to variable burst pressure values and potentially an inaccurate measure of seal patency.

\textbf{Biophysics of sealed tissue and its relationship to burst pressure testing}

All tissues in the body are comprised in part of collagen. However, there is considerable variation in the type and in the amount depending on the physiologic function of the structure [51, 52]. Our group and others have studied C/E content and its impact on seal quality in depth in vasculature [21-23]. Collagen and elastin are the major components in a vascular seal. C/E has been shown to vary in physiologically discrete vessels, and as early as 1966, physiologists described differences in C/E and corresponding regional variation in elasticity of arteries originating from functionally distinct vascular beds in a canine model [51]. In the \textit{in vivo} porcine experiments, our group found similar regional variation in C/E content in a healthy porcine model and demonstrated C/E was associated with arterial burst strength (ABPr), independent of vessel size and origin, following vessel sealing using a bipolar vessel-sealing device (Figure 3) [21].
 Previously the major predictor of burst pressure and vessel-sealing success was thought to be the diameter of the artery involved [21, 22], as the biomechanics of a sealed artery under pressure is often described by Laplace’s law (\( T = rP \), where \( T \) is tension in the vessel wall, \( r \) is the radius of the cylinder [artery], and \( P \) the pressure of the fluid within the artery). However, Laplace’s law does not take into account the elasticity of the individual constituents of the vessel, which are defined by Young’s modulus. Young’s modulus describes the elasticity of a material and is defined as the
tensile stress a material can withstand before breaking divided by the extensional strain
the material can withstand before breaking [52, 53]. The Young’s modulus for elastin is
3.9 x 10 dynes/cm and is 1 x 10 dynes/cm for collagen [52].

\[
E \equiv \frac{\text{tensile stress}}{\text{extensional strain}} = \frac{\sigma}{\varepsilon} = \frac{F/A_0}{\Delta L/L_0} = \frac{FL_0}{A_0 \Delta L}[50, 51]
\]

Where:
- \( E \) is the Young’s modulus (modulus of elasticity)
- \( F \) is the force exerted on an object under tension
- \( A_0 \) is the original cross-sectional area through which the force is applied
- \( \Delta L \) is the amount by which the length of the object changes
- \( L_0 \) is the original length of the object

At physiologic pressure, elastin fibers are recruited and allow some deformation
of the vessel. However, at higher, supraphysiologic pressures, collagen fibers are
recruited and, with a Young’s modulus several hundred times less than that of elastin,
the tension in the vessel wall rises rapidly with little additional deformation until a
critical pressure is reached and the vessel bursts. Thus, considering Laplace’s law, one
would predict that vessel diameter would have a large impact on the maximum burst
pressure.

Yet in our experiments over more narrow ranges of vessel sizes (2-5 mm), it has
become apparent that although diameter is important, a critical determinant of the
strength of an arterial seal using EBDs is the C/E content of the arterial wall. Previously
published data by our group confirmed C/E in the sealed vessel was a major contributor
to seal quality variability in both healthy and atherosclerotic porcine vasculature [21, 22].
The effect of collagen content on burst pressure was also confirmed by our group in
human vasculature in a cadaver model [23]. Other investigators have extended our
observation in terms of reduced efficacy (burst pressure) observed in veins with lower
collagen content compared to arteries [24]. This novel finding contradicts the long-held
notion that vessel diameter is the most important predictor of burst pressure. These
studies have demonstrated that the burst pressure of a vessel is a function of both the quality of the seal and the innate elasticity of the vessel.

Variation in C/E affects not only the amount of robust material incorporated into the seal i.e. more or less collagen, but is also a critical determinant of the distensibility or the overall elasticity of tissue. The ratio of these two proteins in the vessel wall and their interaction (Figure 4) play a key role in determining the overall pliancy of the vessel and the wall tension when the vessel is distended [52]. Given the biochemical and mechanical means by which seals are formed, the ratio of the specific proteins (C/E) that undergo denaturation provides a logical explanation of why C/E is such a good predictor of seal quality, and thus burst pressure. Conversely, vessel elasticity and distensibility is a function of the biophysical properties of the interrelated constituents of the artery, most notably the endothelium, smooth muscle, elastin and collagen. Each of these constituents has a particular Young’s modulus value, and each contributes to the elasticity of the vessel to varying degrees [52]. Other variables which alter the vascular collagen and elastin content such as disease state, smoking status and age are clinically important predictors of vascular pliancy and distensibility [54].

Figure 4. (a) Interaction of elastin (black) and collagen (blue) in Masson Trichrome stained porcine renal artery; (b) SEM of relaxed and stretched arterial lamellar unit.
Cystic duct vs. vasculature

The cystic duct has similar morphologic features to vasculature, including a tubular distensible structure, and a lamina comprised of collagen and elastin fibers although the exact ratio and overall Young’s modulus of the cystic duct is unknown. This similarity likely contributed to the successful attempts at sealing the duct in the published literature, and suggests that C/E will impact the burst pressure in a manner similar to blood vessels. Changes in human tissue elasticity indicate pathological changes in other disease states such as atherosclerosis, emphysema and breast cancer [55-57]. Inflammation and infection are known to have a substantial effect on the amount of collagen in affected tissue, and these factors may also contribute to an increase in cystic duct diameter, another crucial contributor to burst pressure. Some investigators have shown morphometric and biomechanical remodeling manifest as an increase in wall thickness that correlates with an increase in collagen content in porcine common bile ducts after occlusion [58, 59]. A similar phenomenon may occur in a human cystic duct blocked by a gallstone if the blockage is chronic. Interestingly, the ducts returned to a baseline, normal state after the obstruction was removed.

Adequate vascular collagen content influences device performance and seal quality as measured by the metric of burst pressure [21-24]. It is likely that the same is true for the cystic duct given similar morphology. However, little quantitative evidence exists to date for human cystic duct seals. This study directly addresses the gap in knowledge regarding the quality of seals on human cystic duct tissue created by surgical energy-based devices. The purpose of this study is to compare quantitative burst pressure using US vs. BP energy in human cystic ducts, and to test the impact of cystic duct morphology in terms of diameter and in terms of collagen and elastin content on seal quality.
Characterizing the cystic duct: Impact of C/E ratio on seal strength

One step toward optimization of an EBD for the cystic duct is validation of an appropriate test model and standardized test methodology. Healthy porcine cystic ducts likely differ in terms of inflammation, diameter, collagen and elastin content compared to human diseased cystic ducts. These factors may influence burst pressure and may cause human cystic ducts to behave differently than healthy porcine ducts.

Testing to standards in healthy porcine cystic ducts may result in a device that is designed to work well in the healthy porcine cystic duct model, but may not be as effective in humans and real world clinical scenarios in which seals are performed in the context of disease states such as acute cholecystitis and cirrhosis, which causes a hypertensive biliary tract and ultimately causes a change in the collagen content of the tissue. Development of a robust test methodology and test model may be achieved by first gaining an understanding of the fundamental morphologic characteristics of human cystic ducts. There are currently no data published on the diameter, or the collagen or elastin content of the cystic duct either in a healthy or diseased state.

Further basic study is needed in human cystic ducts to investigate the relationship of collagen content, diameter, disease state and burst pressure in order to determine if there is an association between these variables and seal patency as measured by burst pressure. In addition, a better understanding of the basic relationship between tissue state and type in terms of collagen to elastin ratio, viscoelasticity and seal patency may allow for improvement or expansion of the technology into other new tissue types. Finally, increasing our understanding of human cystic duct characteristics in LC patients may allow for identification of a clinically relevant animal test verification model that closely resembles diseased human tissue.
CHAPTER II
LITERATURE REVIEW

This review of the literature summarizes the published use of US and LS energy for sealing the cystic duct, including bench and animal experimentation and human use.

Literature search strategy

This literature search was conducted in OVID and in PubMed with no limit on publication date. Search terms included: seal, cystic duct, ultrasonic energy, laparoscopic cholecystectomy, bipolar energy, harmonic scalpel, bile leak and Ligasure. Studies in which the cystic duct was not sealed were excluded. All other studies are reviewed below.

Transition from dissection to sealing the cystic duct

Ultrasonic energy was initially used instead of monopolar hook instruments to dissect the triangle of Calot and release the gallbladder off the liver bed. Early authors hypothesized that, since US energy has no risk of capacitive coupling to trocars or to metal surgical clips, it might reduce risk of leak from the cystic duct as a result of perforation from stray current to the clip [60, 61]. Others hypothesized that using US energy as opposed to monopolar energy for dissection might reduce perforation of the gallbladder, improve the sealing of ducts of Luschka on the surface of the gallbladder bed, and reduce pain associated with burns to the liver bed caused by monopolar energy as well as pain caused by bile leak into the peritoneum from perforated gallbladders [60, 61]. Furthermore, they suggested that the use of the device to seal the cystic duct and the cystic artery would allow for use of a single instrument for the entire operation, which would in turn speed up and simplify the surgery. Finally, the possibility of reduced bile leak as a result of clip displacement was also considered a potential benefit and a few
authors began to conduct preclinical research in a porcine model to assess the safety and
effectiveness of energy for sealing the cystic duct.

**Pre-clinical and bench experiments**

In 2001, Matthews et al. at Carolinas Medical Center were the first investigators
to publish bench and animal experiments exploring the use of energy to seal the cystic
duct [18]. The trial was not powered *a priori* and no statistical power was reported. There
were two phases of the experiment. In the first phase, they compared the burst pressure
results of excised human cystic ducts stumps with a 5 mm BP device, a 5 mm US device
and surgical clips. The authors initially collected 45 cystic ducts and sealed 14 with a
surgical clip, 16 with the US device, and 15 with the BP device and then burst pressure
tested them. The burst pressure method is not described in detail. Subsequently they
collected 19 more cystic duct stumps, sealed nine with US, 10 with BP and then did a
histological assessment of thermal spread of the seals.

In the second phase of the experiment, they sealed the common bile duct of three
pigs with the US device and three pigs with the BP device and three with surgical clips.
No cholecystectomy was performed. The authors suggested that the common bile duct is
similar in size to human cystic ducts, however no sizes of either human or porcine ducts
were reported and no references were cited. After six days, the animals were euthanized
and the seals were assessed. The pressure in the common bile duct was measured prior
to euthanasia.

The mean burst pressure of human cystic ducts was 621 mmHg for clips, 428
mmHg for the BP device and 278 mmHg for the US device. No standard deviation or
confidence intervals were reported, and a t test was performed to compare clips to US
and clips to BP. There was a significant difference between the US and the clip pressures
(p=0.007) and there was no significant difference between the BP and the clips (p=0.390),
although the power and Gaussian distribution (and hence suitability of a t test) of this
small sample size is questionable. The mean thermal spread was 3.5 mm for the US device and was 13.4 mm for the BP device (p=0.002), and again, no standard deviation or confidence intervals were reported. The walls of the ducts did not appear to be welded with the US device but were with the BP device. The methods and criteria for measuring thermal spread were not reported.

In the chronic porcine experiments, all the energy seals failed and all of the animals developed bile peritonitis. The three animals with clipped common bile ducts had intact clips at the Day 6 necropsy, and none had evidence of bile leak. The mean back-pressure detected in the common bile duct was 12mmHg (no standard deviation or confidence interval reported). Ultimately they concluded that energy was unsuitable for sealing structures other than blood vessels.

The next group to report on the use of energy to seal the cystic duct in a porcine model was Schulze et al. in Denmark in 2002 [29]. This group used the same 5-mm BP instrument as the Matthews et al but had very different results. They performed laparoscopic cholecystectomy and sealed the cystic duct and artery (as opposed to the common bile duct as in the Carolinas study) in eight pigs. The animals were euthanized after eight days, and assessed for bile leak. All seals were excised and stained with hematoxylin and eosin and histologically assessed for thermal spread and patency. None of the animals demonstrated any evidence of bile leak or peritonitis and all of the cystic duct seals were completely fused. The thermal spread was 0.5 mm, again a very different result than in the study by Matthews et al. The group concluded that a large human clinical trial could be performed based on the results of the animal experiment. They went on to use the BP device to seal the cystic duct during laparoscopic cholecystectomy in a series of 100 patients. (Their paper is summarized in the clinical section.)

The same year, a Hungarian group published the results of a similar experiment. Shamiyeah et al. performed open cholecystectomy on 10 pigs and sealed the cystic duct
and artery [30]. They also measured the diameter of the porcine cystic ducts and found a mean diameter of 1.8 mm (range 1-2 mm). In the first two animals, the investigators used a higher energy setting with 82 watts of power. During these initial surgeries they discovered that this power setting was very high, and suggested that this caused “necrosis” of the ducts. Subsequently they reduced the energy output of the generator to 51 Watts and performed the other 8 operations using this setting. Four of the animals were survived for four days, and the other four were survived for eight days. At necropsy, none of the animals demonstrated peritonitis or any other signs of bile leak. All the cystic artery seals healed without incident. The histological appearance of both groups was similar and they describe thermal spread as “within 2-3 mm of the sealing zone.” The authors concluded that it was “worth performing additional trials” to further assess the safety and efficacy of cystic duct energy seals.

In 2010, two more groups performed experiments to investigate the feasibility of sealing the cystic duct with an EBD. Another group from Carolinas Medical Center performed an experiment very similar to the previous effort from this institution in 2001. Hope et al. sealed porcine common bile ducts in 23 animals [19]. The authors indicated that they chose the common bile duct as opposed to the cystic duct in order to “subject the resultant seals to the maximum conceivable burst load in the form of the largest accessible biliary structure and continued postoperative stress load caused by bile secretion.” The experiment was divided into an acute and chronic group. The acute group contained 11 animals. A midline laparotomy was performed on these animals and the common bile duct was sealed with either clips (n=3), a BP device (n=4), or an US device (n=4). The ducts were excised and immediately burst pressure tested. The mean burst pressure was 646.8 (SD=281.8) for the clipped group, 97.6 (SD=86.6) in the BP group, and 71.7 (SD=89.3) in the US group.

The chronic group contained 12 animals. A midline laparotomy was performed on these animals as well. The physiologic back pressure of the common bile duct was
measured, and then the common bile duct was sealed with either clips (n=4), BP (n=4),
or US (n=4). The animals were closed, and the intention was to survive them for seven
days. In the clip group, two pigs were euthanized on Day 2 due to poor food intake but
both clips were intact. The other two animals survived to Day 7 and the clipped ducts
burst at an average of 1088.0 (SD=922.6). One animal in the BP group was euthanized on
Day 1 due to obvious bile leak, which was confirmed proximal to the seal on surgical
exploration. The other three animals survived until Day 7 but all seals were disrupted
upon dissection and there were no burst pressures measured. In the US group, two
animals were euthanized on Day 3 due to obvious bile leak and leaks from the US seal
were confirmed upon surgical examination. The remaining animals survived until Day 7
but, like the BP group, all seals were disrupted with dissection and there were no burst
pressures attained. Physiologic bile duct pressures were measured in seven pigs at a
mean of 16.1 mmHg (SD=4.1). The authors also measured the common bile ducts and
reported a range of 5.6-6.8 mm, which they suggest is “slightly larger” than a human
cystic duct although no human cystic duct sizes or references were given. They did
reference the earlier 2001 article as the reason for choosing the common bile duct with
regard to size. The authors conclude that energy is unsuitable for closing bile ducts.

The second pre-clinical study published in 2010 took place in Turkey. Kavlakoglu et al. performed a study comparing burst pressure of human cystic ducts closed with surgical clips to those closed with US energy [28]. The investigators burst tested sealed cystic duct stumps from 60 patients after laparoscopic cholecystectomy. It is unclear what the diagnosis was (acute or chronic cholecystitis) as the patients were described as having “symptomatic gallstone disease.” The investigators sealed 30 cystic ducts stumps with the US device in situ with a clip placed proximal to the seal. In the other 30 cystic ducts, two clips were placed in situ, leaving one behind. After removal of the gall bladder, the sealed and clipped cystic duct stumps were burst tested using a methodology that has not been described elsewhere. They used a sphygmomanometer
as opposed to a controlled pressure pump to pump saline through 20-gauge catheters into the cystic duct stumps. The pressure was measured with an arterial line transducer by watching the pressure rise and then writing down the pressure just before it fell due to a burst seal. No power was calculated \textit{a priori} for a difference between the two groups.

There were 39 women and 21 men in the study, reflecting the greater incidence of cholecystitis in women. The mean age in the US group was 47.2 (range=25-80 years) and the mean age in the clip group was 46.7 (range=34-63 years). The mean burst pressure in the clip group was 332.46 (SD=4.62 mmHg) and the mean burst pressure in the US group was 343.06 (SD=4.28). In our experience, it is impossible to attain standard deviations this low with any test method due to the inherent characteristics of tissue. No other authors have recorded a standard deviation this low. The mean burst pressure in the two groups was compared and the authors suggested that the US group was superior to the clip group (p=0.04), however with a difference of only 10 mmHg clinically meaningful “superiority” in terms of burst pressure is unlikely. The authors also suggested that the incidence of gall bladder perforation was lower in the US group compared to the clip group.

An interesting commentary letter by Hope et al., who published the porcine experiment in the common bile duct the same year, followed this paper. Dr. Hope suggested that the “holy grail” of laparoscopic cholecystectomy is the ability to use one instrument to “dissect within Calot’s triangle to obtain the critical view of safety, ligate the cystic artery and the cystic duct, and then dissect the gallbladder from the liver.” He also suggested that the results from the Kavlakoglu study were compelling, but that further study was needed for surgeons to feel comfortable using the device to seal the cystic duct, and to explain the failures in the animal model in which he sealed the common bile duct as opposed to the cystic duct.

In 2011, Kavlakoglu et al. published another, similar study in 90 patients comparing burst pressure measurements in an ultrasound group (n=30), a clip group
(n=30) and a group in which they used a rarely utilized bipolar device called the Plasmakinetic (PK) sealer (n=30) [26]. It appears that the investigators simply added on the PK group to the study described above, publishing in a different journal, because the burst pressures reported for the US group and the clip group are identical. All the same methodology was used, and the burst pressure reported for the PK was 326.56 (SD=4.53). The authors suggested that the HS was superior to both clips and the PK in terms of mean burst pressure, however there was no a priori power calculation.

The last preclinical publication was in 2013 by McVay et al. A group from the Madigan Army Medical Center compared the burst pressure of porcine cystic ducts randomly sealed ex vivo with either standard surgical clips, (n=8) a bipolar device called “Enseal” (n=7) or tied with Endoloops (n=7). Endoloops are sutures threaded through a plastic tube that facilitates easier laparoscopic tying. The authors used a different burst pressure method than has been previously reported in other studies. They cannulated the common bile duct instead of the cystic duct and the entire biliary system was insufflated while submerged in saline. The other end of the cannula was attached to a pressure-monitoring device, and the final pressure was recorded just before a sudden decrease in pressure indicating a burst seal or failure of the tissue. The authors indicated that there was no difference in the cystic duct sized between the two groups, but they did not report the diameters. The mean burst pressure was 432 (range=338-524 mmHg), for surgical clips, 317 (range=262-479) for the Endoloop and 238 (range=118-357) for the Enseal device. The only significant difference was between clips and Enseal (p=0.04). Of note, the Enseal failed to seal the cystic duct in three cases and the Endoloop failed in one case. Interestingly, the sidewall of the cystic duct (35%) and the common bile duct (30%) were the most common failure locations, indicating that the innate tensile strength of the tissue may be lower in some cases than the strength of the seal. The authors concluded that the Enseal device may be a valid option for sealing the cystic duct, but expressed concern over the failure rate of 42% (three failures) for the Enseal device.
Clinical studies

The first clinical study, defined as sealing of the cystic duct in vivo with the autologous seal remaining as the sole method of cystic duct closure, began in 1999 in Rome, Italy, by Huscher et al [17]. The study was published in 2003. Interestingly, this study began before publication of any evidence in animals or excised tissue that energy would be safe and effective in sealing the cystic duct. This prospective non-randomized trial compared complications after cholecystectomy including bile leak rate in 331 patients with cystic ducts sealed with US energy alone to 130 patients with cystic ducts sealed with US energy and backed up with Endoloop. There is no clear indication how the patients were assigned to their study group, although the authors suggest that they used Endoloops on patients who appeared to have very large or very fragile cystic ducts. They also further divided the groups into patients treated by “surgeons in training” (n=176 in the US group, and n=59 in the Endoloop group) and “experts” (n=155 in the US group and n=71 in the Endoloop group). There were 216 women and 115 men in the US group and their ages ranged from 17-91 years. There were 80 women and 50 men in the Endoloop group with ages ranging from 14-89 years. The indications for cholecystectomy were variable. Although the majority was due to chronic or acute cholecystitis, a number of other procedures were also performed along with the cholecystectomy such as hernia repair, Nissen, adrenalectomy, appendectomy, splenectomy, left colectomy, ovarian cyst resection, omentectomy and transduodenal papillotomy. In addition, 17.63% of the patients in the US group and 24.27% of patients in the Endoloop group were treated emergently further adding to the heterogeneity of the surgical population.

One patient died of sepsis after an unrecognized bowel injury from trocar insertion. Complications were defined as minor or major according to the Clavien classification. Complications that were not life threatening and that did not extend hospital stay were classified as Grade I, and minor. All other complications were
considered major on a scale of II-IV. A major complication was defined as one that extended hospital stay and/or required clinical intervention. The US group had nine patients with major complications (2.7%) and 21 with minor complications (6.3%). The Endoloop group had four patients with major complications (3.0%) and 14 (10.7%) with minor complications. Postoperative bile leak occurred in seven patients (2.1%) in the US group and in three patients (2.3%) in the Endoloop group. Reintervention was required for four patients with bile leak from the cystic duct with three of the four failures occurring in the larger US group. The authors concluded that there was no difference between the US group and the Endoloop group in the proportion of patients with cystic duct leak, and that there is a significant learning curve in learning to use US shears to close the cystic duct.

In 2004, the first case series using US energy to seal the cystic duct was performed in the US at a community hospital in Indiana [41]. Dr. Westervelt used US to seal the cystic duct and artery during laparoscopic cholecystectomy in 100 consecutive patients with ages ranging from 17-73 years. All dissection and mobilization of the gallbladder was also achieved using US energy. Two of the patients in the series had very large cystic ducts, and those were backed up with endoloops. There were no bile leaks observed and Dr. Westervelt concluded that US is safe and reliable for completely hemobiliary stasis. In 2005, another Italian surgeon used US energy to seal the cystic duct and artery during LC on a series of 100 patients with chronic cholelithiasis. He excluded patients with acute cholecystitis, jaundice, or pancreatitis. Percutaneous application of a suture to suspend and retract the gallbladder allowed for use of only three ports instead of four. US energy was used for all dissection and for sealing the cystic duct and artery. In eight patients, a four trocar was needed to facilitate safe dissection of the gallbladder. In two cases, the surgeon was unsure if the cystic duct sealed and used a clip to back up the seal. None of the patients experienced a cystic duct leak but one patient had a bile leak from a Luschka bile duct on the gallbladder bed. The
surgeon concluded that the three-port technique with US sealing of the cystic duct and artery was safe and effective in this population with minor disease and little to no inflammation.

Citing the successful outcomes observed in the three prior clinical papers, Bessa et al. conducted a randomized trial in 2008 at the University of Alexandria in Egypt [62]. After ethics board approval, the authors randomly assigned 60 LC patients to have their cystic duct and artery sealed with US energy, and 60 patients to have their cystic duct sealed and cut with traditional clip and cautery (CC). LC was performed using the traditional 4 trocar method. Patients with common bile duct stones, acute cholecystitis, previous upper abdominal surgery, suspected malignancy and pregnant patients were excluded. Demographic data including age, sex, BMI and comorbidities were collected. Operative time and complications, including incidence of gallbladder perforation, were recorded. Dissection was performed at a power setting of “5” on the US device, which allows for more cutting and less coagulation. In the US group, sealing of the cystic duct and artery was performed at a power setting of 2, which allows for more coagulation prior to cutting. The groups were not significantly different in terms of demographic variables. There were 95 females and 25 males total. Mean age was 41.5 (SD=10.3) in the US group and 42.5 (SD=11.4) in the CC group. Of the patients, 58.3% were obese with a BMI >30 in the HS group and 68% were obese in the CC group. The CC group experienced a higher incidence of gallbladder perforation relative to the US group at 30 vs. 10% (p=0.002). Mean operative time was significantly different between the two groups with the operation completed in a mean of 32.1 (SD=7.6) minutes in the US group and in 40 minutes CC group, (p=0.00) without gallbladder perforation. With gallbladder perforation the times were not significantly different at 59.2 (SD=14) minutes and 61.9 (SD=12.2) minutes for the US and CC groups respectively. Postoperative complications included two patients in the US group and three patients in the CC group with a port site infection, and one patient in each group with a chest
infection. There were no bile leaks in either group. The authors concluded that US energy is safe and effective for sealing the cystic duct.

In 2010, there were five papers published describing the use of US energy to seal the cystic duct during LC, and all except one originated in Egypt. The first two papers were based on work from the same group at Mansoura University. Kandil et al. prospectively randomized 70 otherwise normal patients to LC performed with traditional CC and 70 patients to LC performed with US energy including dissection and sealing the cystic duct and artery [63]. The groups were evenly matched in terms of demographic variables. There were 30 men in the US group and 29 men in the CC group. The mean BMI was 28.64 (SD=4.46) in the US group and 28.14 (SD=3.87) in the CC group. The groups were also evenly matched in term of comorbidities. Twelve patients in the US group and 13 patients in the CC group had diabetes. Seven patients in the US group and seven patients in the CC group had hypertension, 15 patients in the HS group and 14 patients in the CC group had cirrhosis, and 15 patients in the HS group and 13 patients in the CC group were smokers.

The two groups were compared in terms of intraoperative blood loss, bile spillage, duration of the operation, conversion rate, amount of drainage and hospital stay. The US group was statistically superior on every variable except for conversion rate. Postoperative complications including pulmonary complications, port site infection, bile leak, body temperature at 24 and 48 hours, nausea at 24 and 48 hours, and vomiting at 24 and 48 hours were also tracked in both groups. There was no statistical difference in any of the parameters tracked. Finally, pain location (incisional or shoulder), and pain intensity was studied as either a binomial (presence of pain: yes/no) variable or measured with the visual analogue scale (VAS) at 12, 24 and 48 hours. The US group had significantly less pain at 12 hours than the CC group, and significantly lower VAS scores at 12 and 24 hours. The location of pain was less often in the shoulder than at the incision site in the US group, possibly due to shorter operation time which
results in less insufflation time. The authors concluded that US energy is a safe and effective method to close the cystic duct and artery with shorter operating time, less gallbladder perforation and a lower rate of conversion from laparoscopic to open surgery.

Following the success of US energy in normal LC patients, El Nakeeb et al. studied the use of US energy in LC in cirrhotic patients at the University of Mansoura Hospital [73]. The authors prospectively randomized 60 patients to US, and 60 patients to CC for cystic duct and artery closure. They recorded demographic variables including: Child-Pugh Class (41 were Class A, 19 were Class B, and 0 were Class C in the CC group, and 46 were Class A, four were Class B, and none were Class C in the US group), sex (there were 35 men and 25 women in the CC group, and there were 42 men and 18 women in the HS group), and presenting symptoms (there were 56 patients in the CC group and 54 in the US group who presented with biliary colic, and there were four patients in the CC group and six patients in the US group who presented with acute cholecystitis). Operative time, hospital stay, time to normal diet, incidence of gall bladder perforation and post operative pain on Day 1 as measured by the visual analogue scale (VAS) were all significantly better in the US group. Conversion rate, proportion of patients requiring blood transfusion, volume of bile drainage, post-operative pain on Day 7 and pain at the surgical site on Day 1 and 7 did not differ between the two groups. The authors conclude that even in this compromised patient population, US energy can safely seal the cystic duct.

A surgeon at Assuit University Hospital explored the use of US energy to facilitate a less invasive double trocar approach to LC using the US device as the single working instrument for dissection and cystic artery and duct sealing. Dr. Redwan randomized 80 patients to the US and double trocar group, and 80 patients to a clip and cautery group using the traditional 3 trocar technique [43]. The surgical indication was described as “symptomatic gallstones.” The groups were roughly evenly matched in
terms of sex with 62.5% female patients overall. Age ranged from < 20 to > 60 years. Operative duration was significantly shorter in the US group with a mean of 16.8 minutes (SD+6.8) relative to the CC group which had a mean of 44.01 minutes (SD+6.47). There was no difference in the proportion of patients who experienced an intraoperative bile spillage with 10% of patients in the US group and 13% of patients in the CC group. One patient with bile leak was identified in the clip and cautery group. The patients in the CC group required more pain medication than those in the US group. The mean hospital stay was significantly different between the two groups, although the author suggested that the length of stay may be imprecisely measured, or biased given the unblended nature of the study. The US group remained in the hospital one day after surgery and the CC group remained in the hospital 1.53 days (SD=0.51). Dr. Redwan concluded that US energy achieves complete biliary stasis and shortens operative time relative to traditional clip and cautery. He also concluded that reducing the number of ports may lead to improved cosmesis and less pain.

Gelmini et al. conducted a case series in Italy of 95 LC patients who had their cystic duct sealed with US energy, and compared them retrospectively to 90 historical LC patients who had their cystic duct closed with clip and cautery [42]. Of the patients, 38.9% in the US group were male and 41.11% of the patients in the CC group were male. The mean ages were 52.05 (SD=18.13) and 51.08 (SD=16.41) years in the US and CC groups respectively. Of the patients, 13.65% in the US group had acute cholecystitis and 16.67% of the patients in the CC group had acute cholecystitis. The remainder of the patients in each group had simple cholelithiasis. Of note, the authors recommend using additional ligature on ducts larger than 4 mm in diameter. The median operative time was 60 (range=20-205) minutes in the US group and 85 minutes in the CC group (range=45-150). One patient in the US group had to be converted to open surgery. The post-operative complications collected included “peritoneal fluid collection” with two patients in the US group and no patients in the CC group experiencing this
complication, hemoperitoneum and pleural effusion with one patient in the CC group experiencing each of these complications. The authors suggest that US energy appears safe and effective for sealing the cystic duct, however ducts over 4 mm should have a ligature back up.

Patel et al. conducted a study in 100 LC patients at the Newham University Hospital in London, UK in order to determine if LC could be carried out as a same day procedure using US for dissection and to seal the cystic duct and artery [64]. The authors had no exclusion criteria. The mean age was 46 (SD+13) years, there were 24 men, and the mean BMI was 30 (SD=4). The American Society of Anesthesiologists Scale (ASA) scale was used to determine the relative fitness of each patient for surgery. There were 36 patients at ASA grade 1, 58 at ASA grade 2, and 6 at ASA grade 3. The mean operating time was 27 minutes, the mean blood loss was 5 Ml the mean length of stay was 7.75 days, the mean duration of analgesic use was 7.06 days, and the mean time to return to normal activity was 10.95 days. One patient had a bile leak from the cystic duct stump and there was one conversion to open surgery. The proportion of patients who were fit for release at one day was 65%. The authors concluded that using US energy for all aspects of LC is safe and effective even as a day case procedure.

The first of two studies in which BP energy was used clinically to seal the cystic duct, was published in 2010 by Schultze et al at University of Copenhagen [16]. The same group had previously conducted their own experimental study in 2008 in a porcine model prior to moving to human use. During the trial, 218 LC surgeries were performed, and in 102 of these the cystic duct was closed with BP energy. There was no randomization and it isn’t clear how the groups were determined. Patients who had intraoperative cholangiography were excluded, as were patients with a cystic duct greater than 1 cm in diameter, or shorter than 1 cm in length. There were 32 men in the US group and 39 men in the CC group. The median age was 32 in the US group and 39 in the CC group. There was one complication in each group. In the US group, one
patient developed a leak from a duct of Luschka on the gallbladder bed that required drainage. In the CC group, one patient was readmitted due to pain but no cause was determined. There were no cases of bile leakage in either group. The authors concluded that BP energy was safe and effective but potentially cost prohibitive.

In 2011, Jain et al conducted a prospective randomized trial at Maulana Azad Medical College in New Delhi India [40]. The authors compared outcomes on a number of parameters after the use of US energy vs. traditional CC to seal the cystic duct and artery in 200 LC patients. They excluded patients with impaired liver function, a history of jaundice or pancreatitis, suspected gallbladder carcinoma, common bile duct calculi, pregnant patients, acute cholecystitis or empyema of the gallbladder and those with a common bile duct of more than 5 mm on ultrasonography. The US group fared statistically better in every parameter measured. The duration of stay was 1.89 (SD=0.56) days in the US group compared to 2.52 (SD=0.75) days in the CC group. The fall in hematocrit and hemoglobin was 1.50, (SD=2.50%) and 0.53, (SD=0.52 g%) respectively in the US group and 2.60, (SD=1.295%) and 1.33 (SD=0.85 g%) respectively in the CC group. The time to dissect the gallbladder off of the liver bed was 3.94 (SD=2.07) minutes in the US group and was 7.36, (SD=3.43 minutes in the CC group. VAS on Day 0 was 2.65, (SD=1.04) in the US group and was 4.58, (SD=1.00) in the CC group. VAS on Day 1 was 1.86, (SD=0.59) in the US group and 2.66, (SD=0.66) in the CC group. The number of 50 mg diclofenac tablets consumed on Day 1 was 1.80, (SD=0.59) in the US group and was 2.66, (SD=0.66) in the CC group. There were nine patients in the US group and 18 patients in the CC group who experienced a gallbladder perforation. There were 12 patients in the US group and 31 patients in the CC group who required a drain. In four US patients and in eight CC patients a conversion to open surgery was required due to difficult anatomy. There were no major complications or major bile leaks in either group. The authors concluded that US energy can be used safely in LC for all dissection and for
sealing the cystic duct and artery, and that it is a better tool than traditional clip and cautery as measured by the parameters in their study.

In the second clinical trial published in 2011, investigators from the Johannes Gutenberg University Medical Center used BP energy to seal the cystic duct during LC in 22 children with symptomatic cholecystolithiasis [65]. No cystic ducts larger than 7 mm in diameter were sealed. The patient ages ranged from 7-21 years, with a mean of 14.5. There were 12 boys and 10 girls. Weight ranged from 42-83 kg, with a mean of 58 kg. Four of the children had a previous abdominal surgery. Standard monopolar hook cautery was used for dissection. The authors also utilized a unique “microlaparoscopic” approach in which a 2.4 mm scope was positioned 2.3 cm to the right of the umbilicus, and the umbilicus was used for insertion of the trocar for all working instruments. There was one serious complication. A common bile duct obstruction was identified on postoperative Day 1. Although exploratory laparotomy did not reveal a direct injury to the common bile duct, there were apparent thermal changes to the surrounding tissue associated with the BP device. The authors concluded that the cystic ducts were “closed easily” with BP energy but cautioned that care must be taken to limit the thermal spread to adjacent tissues.

In 2012, Seifarth et al. studied the feasibility single port cholecystectomy in 20 children at Cleveland Clinic [66]. In the first six subjects they used US energy for dissection, and to seal and divide the cystic duct and artery. Although the authors concluded that it was “technically satisfactory” they opted to use Hem-o-lok clips instead for cost containment.

Wills et al. performed the most recent study investigating the use of US energy to seal the cystic duct in 2013 at the Crawford Clinic in Anniston, Alabama [67]. Postoperative complications and readmission rates from 57 LC patients in which US was used to ligate the cystic duct and artery were retrospectively compared to rates in 148 LC patients who had traditional clip and cautery closure of the cystic duct. Thirteen
patients presented with complications at outpatient follow up. Nine were from the CC group and four were from the US group. There was one case of bile leak from the cystic duct in the CC group. There were three cases of bile leak in the US group but these were not from the cystic duct. There were six patients in the US group who required readmission and one in the CC group. The surgeon’s conclusions about the use of US energy were equivocal.

**Summary of literature, importance, and limitations**

Laparoscopic cholecystectomy is one of the most common abdominal operations performed in the western world. Utilizing US or BP energy for dissection and sealing of the cystic duct and artery may reduce the number of patients with bile leak from the cystic duct, from gallbladder perforation, and from ducts of Luschka on the gallbladder bed. In addition, the amount of pain the patient experiences postoperatively may be reduced as a result of the decrease in the amount of bile in the peritoneal space, and as a result of the diminished char to the liver bed that BP and US devices allow compared to traditional monopolar cautery dissection.

The scientific rigor associated with medical device research is on average, far below what is expected in the pharmaceutical industry [68]. Medical devices are often simply tools to facilitate an operation or procedure, whereas drugs are always treatments. Despite this, medical devices can have a large impact on important clinical outcome parameters such as pain, bleeding and bile leak in the case of US and BP devices after surgery.

Even for Type III devices such as pacemakers, radiotherapy devices and angioplasty catheters, which are used for treatment, the size and scope of clinical trials is much smaller than drug trials [68]. A large sample size, the use of placebo interventions and blinding to allocation or intervention are rare. This is due in part to differences in the Food and Drug Administration defined regulatory path to market for devices
compared to drugs and in part to the inherent difficulty of performing surgical or device clinical trials compared to drug trials. Blinding the surgeon as to what device he or she will use is impossible, and blinding the patient as to which device was used, particularly in the case of implants, is often considered unethical, as are placebo interventions. This may lead to substantial bias in the medical device trials.

The literature examining the use of energy to seal the cystic duct during laparoscopic cholecystectomy (LC) is typical of surgical publications describing clinical outcomes after use of a medical device. Although there are four favorable randomized controlled clinical trials, and a total of 1,195 patients have been documented as having their cystic duct successfully sealed with US energy, most surgeons remain unconvinced. Currently US energy is not commonly used in clinical practice to seal the cystic duct in surgical practice in the United States. This may be due to the quality of the reporting of the trials. None of the randomized trials followed consort guidelines. Both the preclinical and clinical literature describing the use of energy to seal the cystic duct is conflicting, with widely variable methodology, contradictory outcomes and poor control of confounding factors such as duct diameter and disease state. None of the papers are hypothesis-driven, and none reported an a priori power calculation or a 95% confidence interval. This may explain the lack of confidence many surgeons express when confronted with the possibility of using energy to create an autologous seal in the cystic duct as the sole source of closure. Finally, although a number of studies have compared BP energy or US energy to clips, none have compared BP energy to US energy as we have in our study. In addition, no prior authors have examined the collagen and elastin content of the cystic duct, or given detailed morphological measurements such as wall thickness and overall diameter as they relate to seal quality or diseases state. This information is critical in defining an appropriate patient population due to the strengths and weaknesses of each energy type. BP devices are typically better sealers, whereas US devices are better dissectors. The goal is to strike a delicate balance and determine the
type of device that is suitable for both needs. The ultimate goal is to improve patient outcomes after LC in terms of postoperative bile leak and pain with the use of BP or US energy and to “do no harm” in altering the traditional clip and cautery method in favor of US or BP energy for this common operation.
CHAPTER III
METHODS AND STATISTICAL PLAN

Research question: Do US and BP energy-based surgical devices adequately seal human cystic duct, and does the energy source impact seal quality given duct diameter and collagen and elastin content?

In order to begin to address our research question, we performed an iterative series of experiments. First, we conducted an acute pilot porcine experiment followed by a survival porcine experiment. Next we moved into human tissue and performed a prospective cohort study using BP energy in excised human cystic ducts. Finally we performed a prospective, randomized trial comparing BP energy to US energy in human cystic ducts. Our rationale and methods for each experiment are detailed below.

Animal studies

Rationale for preclinical work

A porcine model is used for testing vasculature sealing devices for FDA approval [28]. However, it is unclear if pigs are a suitable experimental model for cystic duct sealing. Prior literature describing the use of energy to seal the cystic duct in a porcine model is conflicting. Some authors reported successful outcomes with no bile leak, and other authors reported disastrous outcomes with failure of nearly all seals. The burst pressure methods utilized in our experiments are validated and accepted by FDA. Therefore we wanted to evaluate the porcine model using accepted burst pressure testing methodology.
Objectives for porcine experiments

1. Determine if burst pressures were at an acceptable level in porcine cystic ducts in a pilot acute experiment.

2. Determine incidence of bile leak in a survival porcine model after sealing the cystic duct with BP energy.

3. Determine the burst pressure of cystic ducts sealed with BP energy after seven days of healing, and compare it to the other half of the seal burst immediately after excision. The animal studies are summarized in Figure 5.

![Diagram](image_url)

Figure 5. Porcine study design. (a) Acute porcine study and (b) survival porcine study.
Animal acute experimental methods

Four porcine were utilized for the pilot experiment. Animals were cared for according to the United States Department of Agriculture (USDA) and/or American Association for the Accreditation of Laboratory Animal Care (AAALAC) guidelines. The porcine was prepared and draped for laparoscopic cholecystectomy. General anesthesia was induced with 4.4 mg/kg of Telazol, 0.04 mg/kg of atropine, and 1.5 mg/kg of xylazine (given intramuscularly) and maintained with isoflurane inhalational anesthetic after endotracheal intubation. The lead surgeon dissected the triangle of Calot to expose the cystic duct and artery as well as free the gallbladder from the liver bed. Both the cystic duct and cystic artery were sealed with BP energy to provide hemostasis as well as biliostasis. Roughly 2 cm of cystic duct is required for adequate room on the proximal and distal side of the seal to enable burst testing on the specimen side as well as provide a cystic duct remnant that is not endangering the common hepatic or common bile duct due to thermal spread. Ideally a minimum of a 1 cm margin will be between the BP device and the junction of the cystic duct with the common bile duct. This was followed by the sequence below:

1. Blunt dissection of the connective tissue to clearly expose the cystic duct.
2. Verification of the cystic duct structure by either demonstrating origin at the gallbladder neck or termination at the common bile duct.
3. Apply a single activation of the BP instrument (2 bar setting) in an appropriately sized portion of the duct and 5mm from the common bile duct.
4. Remove the specimen (gallbladder and portion of cystic duct).
5. Using a caliper, measure the outside diameter of the cystic duct.
6. Burst the specimen side of the cystic duct seal. (Burst pressure methodology is described in detail later in this chapter.)
7. Record the pressure reading in the appropriate location of the data sheet.
Animal survival experimental methods

Ten animals were utilized for the survival experiment. Animals were cared for according to the United States Department of Agriculture (USDA) and/or American Association for the Accreditation of Laboratory Animal Care (AAALAC) guidelines.

The porcine was prepared and draped for laparoscopic cholecystectomy. General anesthesia was induced with 4.4 mg/kg of Telazol, 0.04 mg/kg of atropine, and 1.5 mg/kg of xylazine (given intramuscularly) and maintained with isoflurane inhalational anesthetic after endotracheal intubation. The lead surgeon dissected the triangle of Calot to expose the cystic duct and artery as well as free the gallbladder from the liver bed. Both the cystic duct and cystic artery were sealed with BP energy to provide hemostasis as well as biliostasis. Roughly 2 cm of cystic duct is required for adequate room on the proximal and distal side of the seal to enable burst testing on the specimen side as well as provide a cystic duct remnant that is not endangering the common hepatic or common bile duct due to thermal spread. Ideally, a minimum of a 1 cm margin will be between the BP device and the junction of the cystic duct with the common bile duct. This was followed by the sequence below:

1. Blunt dissection of the connective tissue to clearly expose the cystic duct.
2. Verification of the cystic duct structure by either demonstrating origin at the gallbladder neck or termination at the common bile duct.
3. Apply a single activation of the BP instrument (2 bar setting) in an appropriately sized portion of the duct and 5mm from the common bile duct.
4. Remove the specimen (gallbladder and portion of cystic duct).
5. Burst the specimen side of the cystic duct seal.

6. Record the pressure reading in the appropriate location of the data sheet.

7. Necropsy: At postoperative Day 7 the animals were subjected to the same general anesthesia protocol described previously. Surgical exploration and removal of the cystic duct remnant was performed. The remnant was burst tested.

Human clinical trials

Rationale for two clinical trials

Due to the paucity of data in the literature on the burst pressure of human cystic ducts, particularly for BP seals, there was no prior data on which to power a prospective randomized trial. Therefore, two distinct clinical trials were conducted to answer this research question. The first trial was a prospective cohort trial using BP energy to seal excised human cystic ducts. The second trial was a randomized controlled trial comparing US energy to BP energy in excised human cystic ducts. The methods for the two trials overlap, but there were two different trial designs. The first trial was a pilot trial and was conducted because although we had preclinical experimental data in a porcine model, there was no evidence that the porcine model was clinically relevant, and there was no published data describing the burst pressure in human cystic ducts sealed with BP energy. As a result, we had no reliable data on which to power the prospective randomized trial. The prospective cohort study is summarized in Figure 6.
Figure 6. Pilot prospective cohort study design.

Objectives prospective cohort study

Our objective in the pilot prospective cohort study was to evaluate the feasibility of sealing the cystic duct with BP energy in patients with chronic cholecystitis, including determining the distribution of cystic duct burst pressures, quantifying the range of cystic duct diameters and wall thicknesses, and understanding if the predictors of vascular burst pressure are also associated cystic duct burst pressure. Our specific objectives were as follows:

1. Characterize the distribution of burst pressure for cystic ducts sealed with BP and estimate the proportion of seals that burst below 45 mmHg. The standard metric adopted for vascular testing of a burst pressure is three times the normal systolic pressure. Given the similarity in gross morphology between the cystic duct vasculature, we adopted a similar standard. The basal physiologic pressure
of the cystic duct is 15 mmHg; therefore we used 45 mmHg as the cutoff for acceptable burst pressure.

2. Determine the relationship between cystic duct diameter and burst pressure. Given the consistent relationship of diameter to vascular burst pressure in previously published studies, we hypothesize that increasing inner or outer diameter will be associated with lower burst pressure.

3. Determine the relationship between burst pressure and wall thickness of the cystic duct. Adequate jaw pressure is fundamental to the function BP sealing devices. Given that wall thickness may make it more difficult to compress the cystic duct relative to vasculature, we hypothesize that increasing wall thickness (defined as $\frac{\text{outer}-\text{inner}}{2 \text{diameter}}$) will be associated with lower burst pressure.

4. Determine the relationship between cystic duct burst pressure and patient characteristics. Given the association between complications including common bile duct injury after LC with BMI, age and male sex, we hypothesize that increasing BMI, age or male sex will be associated with lower burst pressure.

**Methods prospective cohort study**

The study was approved by the Colorado Multiple Institutional Review Board under COMIRB number 09-0049. All patients over the age of 18 years who were in the University of Colorado Hospital for non-emergent laparoscopic cholecystectomy for treatment of chronic cholecystitis were offered informed consent for the study. Forty consecutive patients consented and were included in the trial over a period of 18 months. A single surgeon performed all the operations and obtained all burst pressure measurements. An independent clinical pathologist confirmed diagnosis as well as the severity of the cholecystitis with routine microscopic examination of the excised
gallbladder and cystic duct margin. Demographic information including sex, diagnosis, body mass index (BMI), and age were recorded at the time of surgery.

After standard laparoscopic cholecystectomy the excised gallbladder with the cystic duct stump was taken to a back table in the operating room. The cystic duct stump outer and inner diameters were measured using digital calipers. The wall thickness was defined as the outer diameter minus the inner diameter divided by two. The percent skeletonization of the cystic duct stump was estimated by the surgeon as 25, 50, 75 or 100%. In addition, the surgeon noted whether a one or two bite seal was required. The cystic duct stump was sealed with a commercially available radiofrequency bipolar sealing device with five mm wide jaws (LigaSure™, Covidien, Boulder, Colorado).

Cystic duct burst pressure (CdBPr) was then determined using the same method as previously reported for arterial seals. A blunt-tipped metal catheter attached to pressure tubing was inserted into the open lumen of the duct and secured to the catheter with an iris clamp. The pressure tubing was in turn attached to a digital Fluke pressure monitor to record maximal intraluminal pressure (mmHg). A Cole-Parmer automated injection system was used to inject 0.9% (w/v) saline into the arterial lumen at a rate of 7.5 ml/min via a Y-connector in the pressure tubing. The maximal pressure was recorded as CdBPr for a given vessel seal. The method is described in detail later in this chapter.

**Statistical methods prospective cohort study**

Descriptive statistics were calculated for patient demographics and all experimental variables. For aim 1, the proportion of seals that burst below 45 mmHg
and a Wilson 95% confidence interval for the proportion were calculated. In addition, histograms of burst pressures were created to describe the distribution of burst pressures. Multiple linear regression models with the outcome of burst pressure were used to evaluate the hypotheses in aims 2-4. For aim 1, predictors included inner and outer diameter; for aim 2, wall thickness; and for aim 3, age, sex and BMI. For each model an overall 0.05 level F-test was used to determine if the predictors in the model explained a significant proportion of the variation in burst pressure. If significant, two-sided 0.05 level Wald t-tests were used to evaluate the association between each predictor and burst pressure.

**Prospective randomized controlled trial**

A prospective randomized experimental trial was conducted to compare the burst pressures of excised human cystic duct stumps sealed with US and BP energy. A number of clinical trials have found that US energy can safely seal the cystic duct, however, there is less evidence for BP energy. As BP energy typically creates more robust seals in other tissue types, we hypothesized BP energy could also adequately seal cystic ducts and that burst pressures of cystic ducts sealed with BP energy were non-inferior to burst pressures from ducts sealed with US energy.

The specific aims for the randomized controlled trial are as follows:

**Specific aim 1**

Compare the mean burst pressure of human cystic ducts successfully sealed with US energy or BP energy.
Inclusion/Exclusion: All patients ≥18 yrs. Dx with Cholecystitis scheduled for elective laparoscopic cholecystectomy at UCH

Informed Consent Process

Randomization to US or BP

Randomization to US or BP

Surgery Excise Gall bladder cystic duct stump

Surgery Excise Gall bladder cystic duct stump

Measure Cystic Duct inn and outer diameter

Seal Cystic Duct Stump

- US
- Bipolar

- Burst pressure

US sealed specimen

Histology C/E

BP sealed specimen

Histology C/E

Figure 7. Study design randomized controlled trial.

The primary hypothesis is that the mean burst pressure will be similar for BP and US devices. Cystic ducts sealed with BP energy will burst at a mean pressure no more than 30% lower than the US device.
Specific aim 2

Evaluate the relationship between collagen and elastin content, cystic duct diameter, and burst pressure.

Hypothesis 2 is that controlling for cystic duct diameter, ducts with higher C/E will burst at higher pressures.

Specific aim 3

This aim is exploratory. Determine other tissue and patient characteristics that may relate to burst pressure and/or collagen elastin content. Explore the role of comorbidities, duct diameter, age, BMI, sex and burst pressure and collagen/elastin ratio.

Hypothesis 3 is that patients with older age, higher BMI, and male sex will have lower C/E than younger, thinner, female patients.

Hypothesis 4 is that patients with older age, higher BMI and male sex will have cystic ducts that burst at lower pressures than younger, thinner female patients.

Experimental methods: Randomized controlled trial

The study was approved by the Colorado Multiple Institutional Review Board under COMIRB number 14-0064. All patients over the age of 18 years who were in the University of Colorado Hospital for non-emergent laparoscopic cholecystectomy for treatment of chronic cholecystitis were offered informed consent for the study. Forty consecutive patients consented and were included in the trial over a period of 18 months. A single surgeon performed all the operations and obtained all burst pressure measurements. An independent clinical pathologist confirmed diagnosis, as well as the severity of the cholecystitis, with routine microscopic examination of the excised gallbladder and cystic duct margin. Demographic information including sex, diagnosis, body mass index (BMI), and age were recorded at the time of surgery.
After standard laparoscopic cholecystectomy the excised gallbladder with the cystic duct stump was taken to a back table in the operating room. The cystic duct stump outer and inner diameters were measured using digital calipers. The wall thickness was defined as the outer diameter minus the inner diameter divided by two. The percent skeletonization of the cystic duct stump was estimated by the surgeon as 25, 50, 75 or 100%. In addition, the surgeon noted whether a one, or two bite seal was required. The cystic duct stump was sealed with a commercially available radiofrequency bipolar sealing device with five mm wide jaws (LigaSure™, Covidien, Boulder, Colorado).

Cystic duct burst pressure (CdBPr) was then determined using the same method as previously reported for arterial seals. A blunt-tipped metal catheter attached to pressure tubing was inserted into the open lumen of the duct, and secured to the catheter with an iris clamp. The pressure tubing was in turn attached to a digital Fluke pressure monitor to record maximal intraluminal pressure (mmHg). A Cole-Parmer automated injection system was used to inject 0.9% (w/v) saline into the arterial lumen at a rate of 7.5 ml/min via a Y-connector in the pressure tubing. The maximal pressure was recorded as CdBPr for a given vessel seal. The method is described in detail later in this chapter.

**Statistical methods and analysis plan: Randomized controlled trial**

**Power and sample size calculation for randomized controlled trial**

This trial is powered on the primary hypothesis: “Cystic ducts sealed with BP energy will burst at a mean pressure no more than 30% lower than those sealed with US energy.” Alternately, this hypothesis may be stated that the mean ln(burst pressure) of cystic ducts sealed with BP energy will be no more than 0.357 ln(mmHg) lower than for cystic ducts sealed with US energy. A two-sided independent Satterthwaite (unequal variance) t-test will be used to compare mean ln(burst pressure) between the US and BP
groups. Based on the pilot study, a standard deviation of 0.5 was expected for ln(burst pressure) in the BP group. Using the US device, standard deviations for cystic duct burst pressures have ranged from 4 to 47 in the literature, with means ranging from 168 to 343 mmHg [26, 27]. Standard deviations on the natural log scale were approximated based on the coefficient of variation and range from 0.012 to 0.247. Therefore, standard deviations of 0.5 and 0.25 were assumed for the BP and US group respectively in sample size calculations. Setting the type 1 error rate at 5%, 21 subjects per group are needed to exceed 80% power to detect a difference of 0.357 ln(mmHg) between the groups using the two-sided Satterthwaite t-test (Table 1).

### Table 1. Power for a Given N

<table>
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<tr>
<th>N per Group</th>
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<tr>
<td>20</td>
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<td>0.850</td>
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**Specific aim 1 analysis**

A 2-sided 0.05 level independent Satterthwaite t-test will be used to test the primary hypothesis that the mean ln(burst pressure) of cystic ducts sealed with BP energy will be no more than 0.357 ln(mmHg) lower than for cystic ducts sealed with the US device.

**Specific aim 2 analysis**

Multiple linear regression will be used to determine if collagen to elastin ratio is associated with burst pressure controlling for cystic duct diameter and device type used to seal the duct. Ln(burst pressure) will be modeled in a multiple linear regression framework including collagen to elastin ratio, duct diameter and device (BP vs. US) as predictors. A 0.05 level overall F-test will be performed to determine if these covariates
explain a significant proportion of the variability in ln(burst pressure). If the overall F-test is significant, a 0.05 level 2-sided Wald t-test will be used to test if the regression coefficient for C/E is significantly different from 0. A significant result would indicate that C/E is associated with burst pressure, controlling for diameter and device.

**Specific aim 3 analysis**

To assess hypothesis 3, that patients with older age, higher BMI and male sex will have lower C/E, C/E will be modeled in a multiple linear regression framework, including age, BMI, and sex as covariates. Similar to the analysis plan for hypothesis 2, a 0.05 level overall F-test will be performed to determine if these covariates explain a significant proportion of the variability in C/E. If the overall F-test is significant, 0.05 level Wald t-tests will be used to test if the regression coefficients for age, BMI and sex are significantly different from 0. Scatterplots of residuals will be evaluated to determine if a transformation of C/E (such as a natural log transformation) is required to meet the assumptions of linear regression.

To assess hypothesis 4, that patients with older age, higher BMI, and male sex will have cystic ducts that burst at lower pressures, ln(burst pressure) will be modeled in a multiple linear regression framework, including age, BMI, sex and device used to seal the cystic duct as covariates. Again, a 0.05 level overall F-test will be performed to determine if these covariates explain a significant proportion of the variability in ln(burst pressure). If the overall F-test is significant, 0.05 level Wald t-tests will be used to test if the regression coefficients for age, BMI and sex are significantly different from 0.

In addition, exploratory analyses will be conducted to determine other predictors of burst pressure, collagen content, elastin content and C/E using multiple linear regression. Backwards selection with a p-to-stay of 0.1 will be used to choose final models for each of these four outcomes. For ln(burst pressure) potential predictors will
include: device, duct diameter, C/E, collagen content, elastin content, age, BMI, sex, smoking status (current, former or never), diabetes status and whether the device needed to be applied once or twice to create a seal. For collagen, elastin and C/E, potential predictors will include duct diameter, age, BMI, sex, smoking status (current, former or never) and diabetes status.

**Inclusion and exclusion criteria and study population**

Subjects undergoing cholecystectomy for symptomatic cholelithiasis were recruited in the principal investigators’ pre-operative clinic at the University of Colorado Hospital. All subjects will give informed consent and will be 18 years and older. Patients with emergent, acute cholecystitis were excluded.

**Clinical application, randomization procedure and specimen collection**

Written informed consent was obtained for all subjects prior to enrollment. Subjects who are scheduled to undergo laparoscopic cholecystectomy will be randomized on the day of surgery to have their excised cystic duct stump sealed with either a radiofrequency sealing device (LigaSure™, Maryland, Covidien, Boulder, CO) or an ultrasonic sealing device (Sonicision™, Covidien, Boulder, Colorado) on a back table after their surgery. The randomization process will occur via random number generation with allocation blinded until the day of the surgery. The inner and outer diameter of cystic duct will be measured with calipers. The excised and sealed cystic ducts will be burst tested and the maximum pressure before bursting will be recorded in the operating room. After burst testing the sample will be divided in two. One half will be frozen for measurement of hydroxyproline (collagen content) and of elastin content. The other half will be processed routinely to microslides and stained with hematoxylin and eosin (H&E) and modified trichrome stains and reviewed by an independent pathologist for cholecystitis severity. Demographic data including age, diabetic status,
history of coronary artery disease, history of cerebral vascular event, BMI, sex and indication for surgery will be collected.

**Histological analysis**

Hematoxylin and Eosin (H&E) and Masson’s trichrome staining were performed on all samples. H&E is a standard stain utilized on all virtually all clinical samples, and has historically been the method used to measure seal quality in vasculature. Modified trichrome is considered a “special stain” and was performed to clearly identify both denatured and native collagen, elastin, and smooth muscle [69]. Collagen is stained blue, elastin black and smooth muscle red. Denatured collagen is stained a deep purple as a result of dye interaction with ions exposed during the loss of organized, parallel alignment of collagen fibers [70]. Immediately following duct diameter measurement, ducts were divided into two or more samples. The sample was placed into neutral-buffered formalin and stored at –4°C overnight prior to dehydration with serial ethanol cycles and embedding in paraffin. Paraffin embedded tissue was then cut into 5-µm sections, mounted on slides and deparaffinized. Serial sections were then stained using H&E or Masson’s trichrome stain.

**Burst pressure methods**

**Equipment and materials**

A burst test system consists of:

- Cole parmer pump (Figure 8)
- Fluke 717 100G pressure calibrator (pressure gauge) (Figure 9)
- Burst test fixture (Figure 10)
- Blunt-tipped needle (Figure 11)
• Luer lock 20cc syringe and surgical tubing (Figure 12) fitted with a T connect and luer locks as shown

• Liquid: De-ionized water should be used to prevent sensor of pressure gauge from damage

• Sealed Blood Vessel: The distance from the edge of seal to the end of the blood vessel should be at least 4 mm

Figure 8. Cole Parmer pump.

Figure 9. Fluke pressure calibrator (pressure gauge).
Figure 10. Burst test fixture.

Figure 11. Blunt-tipped needles with gauge ranging from 14 to 30.

Figure 12. Syringe and Y-shaped surgical tubing.
Procedure

Assembling and preparing the burst test system

Connect one luer-lock end of the Y-shaped tubing to a syringe and fill the syringe and tubing with de-ionized water (Figure 13). Be sure to minimize air bubbles in the syringe and tubing.

Put the syringe on the pump and turn on the pump; while the pump pushes water through tubing at a slow rate connect the second end of tubing to the pressure gauge and the last end to a needle (use a needle with gauge appropriate to vessel size). Place the tubing with the needle in the groove of burst test fixture. Stop the pump.

Turn on the pressure gauge. Choose mmHg as unit for pressure.

Performing burst test

Choose a needle appropriate to the vessel size. Connect the needle to the tubing.

Figure 13. Assembly of burst test system.
Start the pump for few seconds until water flows through needle tip, and zero the pump. This step is necessary to avoid air inside needle and to remove back pressure from the burst pressure measurement. Stop the pump.

Select flow rate on the pump according to needle gage (see Table 2 below).

Gently slide needle into sealed blood vessel through its opening. Do not let the needle tip touch the sealed wall of vessel; if this is the case, the reading pressure will be artificially high but it does not represent true value.

If the vessel has one or more bifurcations, those bifurcations need to be sealed with a clip (see Figure 14).

### Table 2. Needle Gauge and Applied Liquid Flow Rate

<table>
<thead>
<tr>
<th>Needle Color</th>
<th>Needle Gage</th>
<th>Flow Rate (ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lavender</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>Clear White</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>Peach (translucent)</td>
<td>26</td>
<td>50</td>
</tr>
<tr>
<td>Dark Pink / Red</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Orange</td>
<td>23</td>
<td>100</td>
</tr>
<tr>
<td>Baby Blue</td>
<td>22</td>
<td>100</td>
</tr>
<tr>
<td>Light Pink / Dark Purple</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Bright Green</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>Forest Green</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>Black</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td>Brown</td>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>Army Green / White</td>
<td>14</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure 14. Seal bifurcation using a clip.

Slide the seal through the iris of the burst test fixture and close iris gently (Figure 15).

Figure 15. Close iris on seal.

Start the pump and watch the seal until the seal bursts and determine if it bursts at the edge of seal or through the seal. Stop the pump.

Obtain the maximum reading of pressure from pressure gauge, record onto a data sheet, then hit the ”clear” button (or hold min/max) to erase the number and be ready for the next seal.

Remove the seal from needle and discard it into the tissue removal bin.
Ensure maximum burst pressure was recorded and note how it burst (through seal or at the edge) onto a data sheet along with other information such as: date, operator’s name, pressure calibrator ID number.

If seal burst through a dissection hole, and it cannot be clipped, then the value cannot be considered the actual burst pressure of the seal, and the appropriate note should be made onto the data sheet (example: ‘re-do’).

If the seal burst through a pin hole adjacent to the seal, then this could be a hole formed from either arcing or jaw pressure and should be considered to be the actual burst pressure of the seal.

At the completion of testing, disassemble burst test system. Remove the needle from the tubing, drain out water from the syringe and tubing, and wash the burst test fixture and needles.

**Collagen (hydroxyproline) quantification assay**

The hydroxyproline assay was performed according to the methods of Edwards and O’Brian [71]. This assay method was modified and streamlined by Cassandra Latimer, myself and others in my group at Covidien as described below.

**Materials used**

- 96-well plate
- Beaker
- Conical tubes
- Filtration bottles
- Floating test tube rack
- Glass bottles (with caps)
- Magnetic stir bar
- Microcentrifuge tubes with filter (0.45 µm nylon filter)
• Microcentrifuge tubes with screw-on caps (1.5 mL volume)
• Microcentrifuge tubes with snap-on caps (1.5 mL volume)
• Parafilm®
• pH strips
• Pipette tips (20-200 µL, 100-1000 µL and 1-10 mL)
• Industrial Sharpie®

**Equipment needed**

• Spectrophotometer
• Automatic pipettor
• Heat plate
• Incubator (capable of 37°C)
• Magnetic stir plate
• Microcentrifuge
• Multichannel pipettes
• Pressure cooker
• Scale (sensitive to 1 mg)
• SpeedVac® system
• Thermocouple
• Timer
• Vortex mixer

**Reagents used**

**Citric acetate buffer**

• Acetic acid (glacial)
• Citric acid monohydrate
• Sodium acetate trihydrate (500g)
• Sodium hydroxide pellets

Hydroxyproline standard stock
• Trans-4 hydroxyproline-L
• Citric acetate buffer (above)

Chloramine-T reagent
• Chloramine-T hydrate, 98%
• Distilled water
• n-Propyl alcohol (1 L)

P-dimethylaminobenzaldehyde (DMBA) reagent
• P-DMBA (100g)
• Perchloric acid, 60% (1 lb.)
• n-Propyl alcohol (1 L)

Other
• Activated Charcoal Powder
• Hydrochloric Acid, 37% (12 M)

Reagent preparation

Citric acetate buffer (pH 6.5)
Prepare before beginning the tissue dissection step.
1. Dissolve following reagents in 75 mL of distilled water in beaker
   a. 1.2 g sodium acetate trihydrate
b. 0.5 g citric acid monohydrate

c. 120 μL acetic acid

d. 0.34 g sodium hydroxide

2. Stir contents with magnetic stir bar until fully dissolved

3. Test pH of solution with pH strips to ensure it is approximately 6.0
   a. If pH is lower than 6.0, add extra sodium hydroxide and repeat test
   b. If pH is higher than 6.0, add extra acetic acid and repeat test

4. Add additional distilled water so the final solution volume is 100 mL

5. Sterilize solution by filtering into a filtration bottle via pressurized vacuum filtration device

Buffer can be stored for one to two months at room temperature.

Hydroxyproline standards

Prepare at start of Day 2, or after SpeedVac® step.

1. Dissolve 100 mg of trans-4-hydroxyproline L in 10 mL of citric acid buffer in a conical tube

2. Vortex until this solution (Solution A) is fully dissolved; Solution A has a concentration of 10 mg/mL of hydroxyproline

3. In a separate conical tube add 1 mL of Solution A to 9 mL of citric acetate buffer

4. Vortex until this second solution (Solution B) is fully dissolved; Solution B has a concentration of 1 mg/mL of hydroxyproline

5. Label 9 snap-top microcentrifuge test tubes and add a set amount of citric acetate buffer and solution B to each, as according to Table 3.

When finished, each test tube should have 1 mL liquid inside of varying hydroxyproline
concentrations.

**Chloramine-T Reagent**

Prepare immediately before use.

1. Dissolve 141 mg of chloramine-T powder in 2.07 mL of distilled water and 2.6 mL of n-propanol in a conical tube
2. Add 5.33 mL of citric acetate buffer
3. Vortex until solution is fully dissolved

**Table 3. Dilution of Hydroxyproline**

<table>
<thead>
<tr>
<th>Test Tube Label (concentration of hydroxyproline at µg/mL)</th>
<th>Citric Acetate Buffer (µL)</th>
<th>Solution B (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>980</td>
<td>20</td>
</tr>
<tr>
<td>40</td>
<td>960</td>
<td>40</td>
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<td>60</td>
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<td>80</td>
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<tr>
<td>150</td>
<td>850</td>
<td>150</td>
</tr>
<tr>
<td>200</td>
<td>800</td>
<td>200</td>
</tr>
<tr>
<td>250</td>
<td>750</td>
<td>250</td>
</tr>
</tbody>
</table>

**p-DMBA Reagent**

Prepare immediately before use and use within an hour.

1. In 15 mL conical tube, dissolve 1.4 of p-dimethyaminobenzaldehyde powder in 7
mL of n-propanol

2. Vortex until powder is evenly distributed (it will not fully dissolve yet)

3. Add 3.0 mL of perchloric acid

4. Vortex until solution is fully dissolved

Solution will be a bright light green color and test tube will feel slightly warm.

Collagen extraction

Tissue dissection

1. Legibly prelabel both the caps and sides of the desired number of screw-on top microfuge test tubes using industrial Sharpie® (number of test tubes is the number of samples being taken)

2. Prepare setup by gathering a glass plate on top of plastic bag filled with ice, filled ice bucket (to store dissected tissue samples), forceps, scissors, labeled test tubes in a test tube rack

3. Dissect a small sample of tissue

4. Zero scale with an empty (prelabeled) test tube and place dissected tissue piece in test tube to weigh

Tissue must weigh between 5 and 10 mg. If the mass is outside this range either add more tissue or remove some from test tube.

1. Record tissue sample number and weight on record sheet, then place test tube in ice bucket

2. Repeat process for all samples
Dissolving tissue
1. Aliquot the required amount of citric acetate buffer in a beaker
2. Add 200 µL of citric acetate buffer to each sample test tube
3. Add 200 µL of hydrochloric acid to each sample test tube

It is recommended to perform this step within a fume hood to avoid toxic fumes from the hydrochloric acid.
1. Screw on tightly and pulse centrifuge all samples to collect the tissue and liquid at the base of the test tube

Tissue hydrolysis
1. Unscrew caps on all samples until the cap is just barely on top (held on by approximately one ring) so that pressure in the test tube can escape but liquid will not evaporate
2. Place all samples in floating test tube rack
3. Place samples in a pressure cooker with boiling water
4. Close pressure cooker lid and wait for pressure to reach 15 psi
5. Once pressure cooker is steadily at 15 psi hold for two hours

Acid evaporation
1. When pressure cooker has cooled, open lid and carefully remove floating test tube rack (it likely will be hot)
2. Dry the outside surfaces of the test tubes with a paper towel.
3. Remove the caps on test tubes
4. Place each test tube in SpeedVac® Concentrator
5. Cool refrigerated vapor trap to -110°C (done by simply turning it on)
6. Run SpeedVac® on high with no heat
SpeedVac® will take four hours to evaporate acid from test tubes. This step should generally be done overnight since test tubes can stably be kept within SpeedVac®. If, after four hours, the liquid is not fully evaporated, re-run test tubes for a shorter amount of time (for sample two hours) until samples are fully evaporated.

Hydroxyproline quantification

Prepare hydroxyproline standard as per reagent preparation instructions.

Filtration.

1. Add 200 µL of citric acetate buffer to each dried sample test tube
2. Screw on all test tube caps and vortex test tubes thoroughly so that the dried component is fully dissolved into the citric acetate buffer
3. Label the required number of filtration microcentrifuge test tubes (with filter spin columns placed inside); there should be enough test tubes to have one for each samples and standards
4. Transfer all liquid in test tube via pipette from each sample or standard from the screw-on (samples) or snap-on (standards) test tube to the corresponding filtration column in a test tube

There should be 200 µL liquid transferred for the samples and 1000 µL transferred for the standards. The liquid should be placed in the filter column within the test tube and not be able to seep into the test tube base.

1. Add a small amount (~1.5 mg, precision here is not important) of activated charcoal on top of each sample in the filter column
2. Tightly cap test tube lids on top of filtration column (this step can be messy)
3. Place test tubes in centrifuge and run for 3 minutes at 11,000 rpm
4. Repeat process of adding buffer to original sample and standard test tubes and then transferring to the filter test tube; this step ensures that no protein components are left in the original container

During the second round the standards will have 200 µL of the citric acetate buffer added to them as well, so only 200 µL of fluid instead of 1000 µL will be transferred to the filter test tubes. After the second round of centrifugation, the filter containers in each test tube can be discarded. Each test tube should have liquid collected at the base.

*Plate pipetting.*

1. Place a 96-well plate on top of plate guide, have plate slanted slightly using a test tube rack.
2. Vortex all test tubes to ensure an even distribution of collagen
3. Carefully add 50 µL of each standard or sample to three wells in a row

This step is fairly difficult because most of the liquid being pipetted is clear, so it is hard to tell what wells have been filled and what wells have not. It is recommended to use pieces of parafilm to cover up wells already filled, and move the pieces of parafilm around to make sure the correct wells are being filled with the correct sample.

*Adding dye reagents.*

1. Prepare fresh Chloramine-T (as per instructions in “reagent preparation”)
2. Pour Chloramine-T into a chemical tray
3. Using multichannel pipette, add 50 µL of Chloramine-T reagent to each well with a sample in it. (It is okay to add the reagent in a well with no samples, but each sample must have Chloramine-T added to it)
4. Cover top of plate fully with parafilm
5. Place plate on top of a shaker plate set to its lowest setting
6. Allow plate to be gently shaken via shaker for 15 minutes
7. Take plate off shaker and remove its parafilm covering
8. Pour freshly prepared p-DMBA to a clear chemical tray
9. Add 50 µL of p-DMBA into each well with a sample in it with the
   multichannel pipette (once again, it is better to add p-DMBA to a well
   without a sample than to miss a well with a sample in it)
10. Put plate in a 37°C incubator for 30 minutes

Do not incubate more than 30 minutes or dye will oversaturate and the plate cannot be
read accurately.

*Plate reading.*

1. After 30 minutes remove the plate from the incubator.

A gradient from yellow to purple should be seen in the wells with the standards in
them, and ideally wells with samples in them should also be a purple shade.

2. Place plate on spectrophotometer surface.
3. Run plate through spectrophotometer set to read at 550 nm wavelength.

**Elastin quantification using BioColor Fastin™ elastin kit**

**Materials used**

- 96-well plate
- Beakers
• Conical Tubes
• Microcentrifuge tubes with screw-on caps (1.5mL volume)
• Microcentrifuge tubes with snap-on caps (1.5 mL volume)
• Pipette Tips (20-200 µL, 100-1000 µL, and 1-10mL)
• Industrial Sharpie®

Equipment needed
• Spectrophotometer
• Automatic pipettor
• Heat block
• Microcentrifuge
• Scale (sensitive to 1 mg)
• Timer
• Vortex Mixer

Reagents
• Fastin™ elastin kit
• Fastin™ dye reagent
• Elastin precipitating reagent
• Elastin standard
• Dye dissociation reagent
• Oxalic acid (1 M)

Elastin assay reagent preparation
• Oxalic acid (0.25 M)
Perform before tissue dissection step.

Combine oxalic acid (1 M) and distilled water in a 1:4 ratio for desired volume in a conical tube i.e. for every one part oxalic acid (1M) added in a container add three parts water.

**Elastin extraction**

*Tissue dissection.*

1. Legibly prelabel both the caps and sides of the desired number of screw-on top microfuge test tubes using industrial Sharpie®
2. Prepare setup by gathering a glass plate on top of plastic bag filled with ice, filled ice bucket (to store dissected tissue samples), forceps, scissors, labeled test tubes in a test tube rack
3. Dissect a small sample of tissue
4. Zero scale with an empty (prelabeled) test tube and place dissected tissue piece in test tube to weigh
   a. Tissue must weigh between 5 and 10 mg
   b. If the mass is outside this range either add more tissue or remove some from test tube
5. Record tissue sample number and weight on record sheet then place test tube in ice bucket
   a. Repeat process for all samples

*Dissolving tissue.*

1. On the record sheet, using mass measurements for each sample calculate how much oxalic acid must be added to each sample; this is done simply by
multiplying the sample mass by 20 which is the volume needed in µL

2. Aliquot required amount of oxalic acid to beaker

3. Add calculated amount of oxalic acid (0.25 M) to each sample; each sample will have a different volume of oxalic acid added depending on its mass

4. Pulse centrifuge samples to ensure all tissue and liquid is collected at bottom of the test tube

5. Place sample test tubes on heat plate (100 °C) for 60 minutes ensuring caps are on loosely

6. Remove all samples from heat block using heat gloves

7. Allow test tubes to cool until at room temperature

8. Centrifuge samples at 7,000 rpm for 10 minutes

9. Transfer liquid extract from original sample test tubes to prelabeled secondary test tubes (try not to disturb or transfer tissue pellet itself; amount of liquid transferred will depend on initial volume added)

Repeat extraction cycle (Steps 2-7) twice for pig tissue and three times for human tissue. All liquid extract should be transferred into the same secondary test tube.

Preparing duplicates.

1. Prelabel the required number (twice the number of samples) of snap-on test tubes for duplicates

Generally it is best to have a labeling scheme of ‘1-A’ and ‘1-B’ to represent the duplicates of sample one.

4. Vortex secondary test tubes to ensure an even distribution of elastin, especially if test
tubes have been in storage.

5. Transfer 20 µL from a sample’s secondary test tube liquid to each of its corresponding duplicates (40 µL is transferred total, split between two test tubes)

**Preparing standards.**

1. Prelabel the required number (likely eight) of snap-on test tubes for samples; standard concentrations of 0, 12.5, 25 and 50 µg α-elastin standard are used, each being tested in duplicate
2. Add 12.5 µL of α-elastin standard to the 12.5 µL standard test tubes
3. Add 25 µL of α-elastin standard to the 25 µL standard test tubes
4. Add 50 µL of α–elastin standard to the 50 µL standard test tubes

Store α-elastin standard in 4°C refrigerator when not in use.

**Elastin isolation.**

1. Aliquot the required amount of Elastin Precipitating Reagent into a beaker
2. Add the Precipitating Reagent to each sample and standard test tube in an equal amount to the amount of liquid already in the test tube i.e. for the samples since 20 µL was added from the extraction step, add 20 µL of Precipitating Reagent to the test tube. For the 12.5 µL standard, add 12.5 µL of Precipitating Reagent, etc.
3. Tightly cap each test tube and briefly invert to ensure complete mixing of liquids
4. Let test tubes stay undisturbed for 10 minutes to allow mixing to occur
5. Centrifuge all test tubes at 11,000 rpm for 10 minutes. This will cause the elastin to precipitate and tightly stick to the base of the test tube
6. Uncap all test tubes and tap out excess liquid onto a paper towel; try to
remove all visible liquid from the test tube, using a Q-tip to remove liquid from the test tube caps as necessary

Elastin should be present as a white substance at the bottom of the test tube.

Elastin dyeing.

1. Aliquot the required amount of Elastin Dye Reagent into a beaker
2. Add 1 mL of the Dye Reagent to each standard and sample test tube
3. Tightly cap the test tubes
4. Vortex test tubes to bring the precipitated elastin into the dye (use highest vortexer settings for about ten seconds, then check base of test tube to see if all elastin has been detached; if not, repeat vortexing steps until elastin appears nearly or fully detached from the base)
5. Organize all test tubes into a test tube rack which can fit on the base of a vortex-shaker, and tape down this test tube rack onto the vortex-shaker to avoid it falling off
6. Gently shake test tubes via the vortex-shaker for at least 90 minutes

Store Elastin Dye Reagent in 4°C refrigerator when not in use.

Elastin-dye separation.

1. Centrifuge all test tubes at 11,000 rpm for 10 minutes
2. Individually uncap test tubes, pour out all dye into a waste beaker, tapping on the test tube to remove liquid.
3. Open test tube cap and, facing the opening downwards, tap the test tube onto
a paper towel to remove remaining dye from test tube

The actual elastin should be seen at the base of the test tube bound to dye. It is necessary to remove all the excess and unbound dye because the amount of dye within the test tube is the indicator for amount of elastin present. Not removing all excess dye will cause an artificial over calculation of elastin.

1. After all visible dye is removed recap test tube and leave inverted on the bench to cause liquid to flow downwards via gravity

Repeat this process (Steps 2-4) for all test tubes. It is suggested to have multiple assistants for this part.

1. Start again with the first inverted test tube (the one which has been inverted the longest) and once again open and tap on a paper towel to remove any dye that has pooled together; test tube should have been left inverted for at least 30 minutes

Repeat Step 5 for all test tubes.

1. Pulse centrifuge all test tubes to collect liquid at base
2. Visually check the test tubes to see that there is very little to no liquid remaining at the base (if there is liquid remaining, continue to tap it out onto a paper towel)

Elastin release.

1. Aliquot the required amount of elastin dissociation reagent into a beaker
2. Add 250 µL of dissociation reagent to each test tube
3. Tightly cap each test tube
4. Vortex test tubes to release the dyed elastin into the solution. Vortex until there is no elastin visibly stuck on the test tube base

This step should cause solution to be tinted a shade of red; the darker the solution, the more elastin present.

Elastin measurement.
1. Transfer the entire 250 µL of solution from each test tube to its designated location on a 96-well plate (see spectrophotometer protocol layout for well locations; in general the standards are added with standard 0 in wells A1 and A2, standard 12.5 in B1, B2, etc., followed with sample 1 added in E1 and E2, etc.)
2. Place plate on spectrophotometer surface
3. Run plate through spectrophotometer set to read at 515 nm wavelength
CHAPTER IV
RESULTS

Results: Porcine experiments

Pilot acute porcine experiment

Cystic ducts from four pigs were sealed in vivo, immediately excised and burst tested (Table 4).

Table 4. Diameter and Burst-Pressure of Porcine Cystic Ducts Immediately After Sealing

<table>
<thead>
<tr>
<th>Pig</th>
<th>Cystic Duct Diameter (mm)</th>
<th>Burst Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.4</td>
<td>657.1</td>
</tr>
<tr>
<td>2</td>
<td>2.1</td>
<td>800.6</td>
</tr>
<tr>
<td>3</td>
<td>2.2</td>
<td>1260.7</td>
</tr>
<tr>
<td>4</td>
<td>5.0</td>
<td>605.3</td>
</tr>
<tr>
<td>Mean</td>
<td>2.9 (SD=1.4)</td>
<td>830.9 (SD=298.2)</td>
</tr>
</tbody>
</table>

Survival porcine experiment

Laparoscopic cholecystectomy was performed without significant complications in 10 Yorkshire pigs. JP drainage was serosanguinous initially after laparoscopic cholecystectomy and was minimal within 24 to 36 hours postoperatively. All pigs survived to exploratory laparotomy at postoperative Day 7. At laparotomy, localized adhesions were noted around the site of JP drain in most pigs without evidence of loculated bile or fluid collections. There were three cystic duct stumps on the gallbladder side, and two cystic duct stumps on the “patient” side that were too short to attach to the burst fixture. Results are represented in Table 5.
Table 5. Burst Pressure Immediately After Sealing on Specimen Side of the Seal (Acute) and After 7 Days of Healing on the Patient Side of the Seal (Survival)

<table>
<thead>
<tr>
<th></th>
<th>Acute Burst Pressure (mmHg) n=5*</th>
<th>Survival Burst Pressure (mmHg) n=8*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>491.8</td>
<td>537.5</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>403.3</td>
<td>343.6</td>
</tr>
</tbody>
</table>

*n does not equal 10 due to short ducts that we could not burst.

**Results: Clinical trials**

**Pilot cohort study with radiofrequency bipolar energy-based device**

Subject characteristics, cystic duct characteristics and burst pressures are presented in Tables 1 and 2. All patients in the study were diagnosed with chronic cholecystitis. Thirty-nine patients had a recorded burst pressure, as one cystic duct stump was too short to burst. One of the 39 cystic ducts sealed burst at 44.6 mmHg, slightly below 45 mmHg (2.6%, 95% Wilson Confidence Interval: 0.1% to 13.2%). A histogram of burst pressure illustrates the slight right skew of the burst pressure distribution (Figure 16).

Inner and outer cystic duct diameters were not significantly associated with cystic duct burst pressure (Multiple R-squared: 0.015, Adjusted R-squared: -0.040, F-statistic: 0.28 on 2 and 36 DF, p-value: 0.76). Similarly, wall thickness was also not related to cystic duct burst pressure (Multiple R-squared: 0.0019, Adjusted R-squared: -0.025, F-statistic: 0.07 on 1 and 37 DF, p-value: 0.79), and there was no relationship
between BMI, sex, or age and burst pressure (Multiple R-squared: 0.014, Adjusted R-squared: -0.071, F-statistic: 0.16 on 3 and 35 DF, p-value: 0.92). Summaries of these models with estimates for the effect of each of the predictors on burst pressure and 95% confidence intervals are presented in Tables 8-10.

**Randomized controlled clinical trial data**

Subject characteristics, cystic duct characteristics and burst pressures are presented in Tables 11-12. None of the ducts burst below 45 mmHg in either the US or BP group.

**Primary hypothesis**

The mean burst pressure will be similar for BP and US devices. Cystic ducts sealed with BP energy will burst at a mean pressure no more than 30% lower than the US device.
Table 6. Pilot Cohort Study: Demographics of Patients

<table>
<thead>
<tr>
<th>Mean (SD), Min-Max or Percent (n)</th>
<th>All Subjects (n=40)</th>
<th>Subjects with Recorded Burst Pressure (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44.3 (15.3), 21-72</td>
<td>44.5 (15.4), 21-72</td>
</tr>
<tr>
<td>BMI</td>
<td>28.8 (6.1), 18-47</td>
<td>28.7 (6.1), 18-47</td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22.5% (9)</td>
<td>20.5% (8)</td>
</tr>
<tr>
<td>Female</td>
<td>77.5% (31)</td>
<td>79.5% (31)</td>
</tr>
<tr>
<td>Severity:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>27.5% (11)</td>
<td>28.2% (11)</td>
</tr>
<tr>
<td>Moderate</td>
<td>65.0% (26)</td>
<td>66.7% (26)</td>
</tr>
<tr>
<td>Severe</td>
<td>7.5% (3)</td>
<td>5.1% (2)</td>
</tr>
</tbody>
</table>

There was not a significant difference in ln(burst pressure) between the two groups (t=1.02, P=0.3). Mean ln(burst pressure) was 0.173 ln(mmHg) higher in the BP group compared to the US group (95% CI: 0.168 lower to 0.514 higher), indicating burst pressures were on average 18.9% higher in the BP group compared to the US group (95% CI: 15.5% lower to 67.2% higher) and ruling out clinically meaningful inferiority of BP energy compared to US. Boxplots of burst pressure and ln(burst pressure) by group are presented in Figure 17.
Table 7. Pilot Cohort Study: Burst Pressures and Cystic Duct Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD), Min-Max or Percent (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burst Pressure (mmHg)</td>
<td>288.7 (139.5), 44.6 – 617.2</td>
</tr>
<tr>
<td>ln(Burst Pressure)</td>
<td>5.5 (0.5), 3.8-6.4</td>
</tr>
<tr>
<td>Inner Diameter of Duct (mm)</td>
<td>3.1 (1.3), 1.2-8.0</td>
</tr>
<tr>
<td>Outer Diameter of Duct (mm)</td>
<td>4.2 (1.6), 2.4-11.0</td>
</tr>
<tr>
<td>Duct Thickness (mm)</td>
<td>0.57 (0.21), 0.35-1.50</td>
</tr>
<tr>
<td>Percent Skeletonized:</td>
<td></td>
</tr>
<tr>
<td>25%</td>
<td>23.1% (9)</td>
</tr>
<tr>
<td>50%</td>
<td>56.4% (22)</td>
</tr>
<tr>
<td>75%</td>
<td>7.7% (3)</td>
</tr>
<tr>
<td>100%</td>
<td>12.8% (5)</td>
</tr>
<tr>
<td>Percent of Jaws Filled:</td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>5.1% (2)</td>
</tr>
<tr>
<td>75%</td>
<td>25.6% (10)</td>
</tr>
<tr>
<td>80%</td>
<td>5.1% (2)</td>
</tr>
<tr>
<td>90%</td>
<td>5.1% (2)</td>
</tr>
<tr>
<td>100%</td>
<td>59.0% (23)</td>
</tr>
</tbody>
</table>

*n does not equal 40 due to one short duct that we could not burst.

There was an outlying subject in the US group that had a very high burst pressure of 700.9 mmHg (subject 45). All other subjects in both groups had burst pressures of less than 463 mmHg. Removing this outlying subject from the analysis, the
Table 8. Pilot Cohort Study: Multiple Linear Regression Model for Aim 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Lower 95% CI Limit</th>
<th>Upper 95% CI Limit</th>
<th>SE</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>323.14</td>
<td>179.89</td>
<td>466.40</td>
<td>70.63</td>
<td>4.575</td>
<td>5.5e-05</td>
</tr>
<tr>
<td>Inner Diameter</td>
<td>-25.23</td>
<td>-184.83</td>
<td>134.36</td>
<td>78.69</td>
<td>-0.321</td>
<td>0.75</td>
</tr>
<tr>
<td>Outer Diameter</td>
<td>10.36</td>
<td>-121.07</td>
<td>141.80</td>
<td>64.81</td>
<td>0.160</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Table 9. Pilot Cohort Study: Multiple Linear Regression Model for Aim 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Lower 95% CI Limit</th>
<th>Upper 95% CI Limit</th>
<th>SE</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>305.10</td>
<td>173.00</td>
<td>437.19</td>
<td>65.19</td>
<td>4.680</td>
<td>3.8e-05</td>
</tr>
<tr>
<td>Thickness</td>
<td>-28.74</td>
<td>-246.39</td>
<td>188.91</td>
<td>107.42</td>
<td>-0.268</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Table 10. Pilot Cohort Study: Multiple Linear Regression Model for Aim 4

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Lower 95% CI Limit</th>
<th>Upper 95% CI Limit</th>
<th>SE</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>267.85</td>
<td>-26.82</td>
<td>562.53</td>
<td>145.15</td>
<td>1.85</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI</td>
<td>1.65</td>
<td>-6.31</td>
<td>9.62</td>
<td>3.92</td>
<td>0.42</td>
<td>0.68</td>
</tr>
<tr>
<td>Age</td>
<td>-0.64</td>
<td>-3.80</td>
<td>2.53</td>
<td>1.56</td>
<td>-0.41</td>
<td>0.69</td>
</tr>
<tr>
<td>Male Sex</td>
<td>9.03</td>
<td>-108.40</td>
<td>126.47</td>
<td>57.85</td>
<td>0.16</td>
<td>0.88</td>
</tr>
</tbody>
</table>
results remain unchanged; there is not a significant difference in ln(burst pressure) between the groups (t=1.4, P=0.16). Removing the outlier, mean ln(burst pressure) was 0.231 ln(mmHg) higher in the BP group compared to the US group (95% CI: 0.097 lower to 0.558 higher) and on average, burst pressure was 26.0% higher in the BP group compared to the US group (95% CI: 9.7% lower to 75.8% higher).

Table 11. Demographics and Descriptive Statistics for RCT, Mean (SD), Min-Max or Percent (n)

<table>
<thead>
<tr>
<th></th>
<th>All Randomized Patients</th>
<th>All Patients with Burst Pressure Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall (n=46)</td>
<td>Overall (n=45)</td>
</tr>
<tr>
<td></td>
<td>US (n=26)</td>
<td>US (n=25)</td>
</tr>
<tr>
<td></td>
<td>BP (n=20)</td>
<td>BP (n=20)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43.1 (13.7) 19-76</td>
<td>42.6 (13.5) 19-76</td>
</tr>
<tr>
<td></td>
<td>44.2 (11.7) 22-64</td>
<td>43.4 (11.2) 22-62</td>
</tr>
<tr>
<td></td>
<td>41.6 (16.2) 19-76</td>
<td>41.6 (16.2) 19-76</td>
</tr>
<tr>
<td></td>
<td>0.54</td>
<td>0.54</td>
</tr>
<tr>
<td>BMI</td>
<td>31.26 (9.6) 19.3-78.1</td>
<td>31.3 (9.7) 19.3-78.1</td>
</tr>
<tr>
<td></td>
<td>31.2 (7.1) 21.3-45.1</td>
<td>31.3 (7.3) 19.3-45.1</td>
</tr>
<tr>
<td></td>
<td>31.3 (12.3) 19.3-78.1</td>
<td>31.3 (12.3) 19.3-78.1</td>
</tr>
<tr>
<td></td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17.4% (8)</td>
<td>15.6% (7)</td>
</tr>
<tr>
<td></td>
<td>15.4% (4)</td>
<td>12.0% (3)</td>
</tr>
<tr>
<td></td>
<td>20.0% (4)</td>
<td>20.0% (4)</td>
</tr>
<tr>
<td>Female</td>
<td>82.6% (38)</td>
<td>84.4% (38)</td>
</tr>
<tr>
<td></td>
<td>84.6% (22)</td>
<td>88.0% (22)</td>
</tr>
<tr>
<td></td>
<td>80.0% (16)</td>
<td>80.0% (16)</td>
</tr>
<tr>
<td></td>
<td>0.73</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Figure 17. Box plots of burst pressure on (a) normal and (b) log scale for the BP and US groups.
Table 11. (continued)

<table>
<thead>
<tr>
<th></th>
<th>All Randomized Patients</th>
<th>All Patients with Burst Pressure Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>82.6% (38)</td>
<td>76.9% (20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90.0% (18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>88.2% (37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>76.0% (19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90.0% (18)</td>
</tr>
<tr>
<td>Current</td>
<td>15.2% (7)</td>
<td>19.2% (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0% (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.6% (7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.0% (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0% (2)</td>
</tr>
<tr>
<td>Former</td>
<td>2.2% (1)</td>
<td>3.8% (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.2% (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0% (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td>Diagnosis:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic Cholecystitis</td>
<td>89.1% (41)</td>
<td>88.5% (23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90.0% (18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>91.1% (41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>92.0% (23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90.0% (18)</td>
</tr>
<tr>
<td>Biliary Dyskinesia</td>
<td>8.7% (4)</td>
<td>7.7% (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0% (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.7% (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0% (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0% (2)</td>
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<tr>
<td>Gallstone Pancreatitis</td>
<td>2.2% (1)</td>
<td>3.8% (1)</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.2% (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0% (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.77</td>
</tr>
<tr>
<td>Co-morbidities:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>78.3% (36)</td>
<td>76.9% (20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80.0% (16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>77.8% (35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>76.0% (19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80.0% (16)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>6.5% (3)</td>
<td>7.7% (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0% (1)</td>
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<tr>
<td></td>
<td></td>
<td>6.7% (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.0% (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0% (1)</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Cerebral Vascular Disease or Stroke</td>
<td>2.2% (1)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0% (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.2% (1)</td>
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<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0% (1)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>17.4% (8)</td>
<td>19.2% (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.0% (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.8% (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.0% (5)</td>
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<td>15.0% (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.82</td>
</tr>
</tbody>
</table>
Table 12. RCT Burst Pressure

<table>
<thead>
<tr>
<th>Burst Pressure (mmHg):</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>US</strong></td>
<td>25*</td>
<td>209.4 (140.9)</td>
<td>184.6 (52.5 to 700.9)</td>
</tr>
<tr>
<td><strong>BP</strong></td>
<td>20</td>
<td>230.8 (102.7)</td>
<td>230.6 (82.7 to 404.8)</td>
</tr>
<tr>
<td>Ln(Burst Pressure (mmHg)):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>US</strong></td>
<td>25*</td>
<td>5.16 (0.63)</td>
<td>5.22 (3.96 to 6.55)</td>
</tr>
<tr>
<td><strong>BP</strong></td>
<td>20</td>
<td>5.33 (0.51)</td>
<td>5.44 (4.41 to 6.00)</td>
</tr>
</tbody>
</table>

*26 were randomized to US – burst pressure was not recorded for 1.

Table 13. Cystic Duct Characteristics RCT

<table>
<thead>
<tr>
<th></th>
<th>Overall (n=45)</th>
<th>US (n=25)</th>
<th>BP (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner Diameter (mm)</td>
<td>2.2 (1.4), 0.6-10.2</td>
<td>1.8 (0.5), 0.6-3.1</td>
<td>2.7 (1.9), 1.2-10.2</td>
</tr>
<tr>
<td>Outer Diameter (mm)</td>
<td>3.0 (1.7), 1.1-13.2</td>
<td>2.3 (0.7), 1.1-4.6</td>
<td>3.6 (2.4), 1.8-13.2</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>0.41 (0.21), 0.15-1.50</td>
<td>0.40 (0.13), 0.20-0.75</td>
<td>0.42 (0.28), 0.15-1.50</td>
</tr>
<tr>
<td>Inflammation:*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>19.6% (9)</td>
<td>15.3% (4)</td>
<td>25.0% (5)</td>
</tr>
<tr>
<td>Minor</td>
<td>8.7% (4)</td>
<td>3.8% (1)</td>
<td>15.0% (3)</td>
</tr>
<tr>
<td>Moderate</td>
<td>58.7% (27)</td>
<td>69.2% (18)</td>
<td>45.0% (9)</td>
</tr>
<tr>
<td>Severe</td>
<td>13.0% (6)</td>
<td>11.5% (3)</td>
<td>15.0% (3)</td>
</tr>
<tr>
<td>Percent Skeletonized:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>6.7% (3)</td>
<td>0</td>
<td>15.0% (3)</td>
</tr>
<tr>
<td>75%</td>
<td>31.1% (14)</td>
<td>24.0% (6)</td>
<td>40.0% (8)</td>
</tr>
<tr>
<td>100%</td>
<td>62.2% (28)</td>
<td>76.0% (19)</td>
<td>45% (9)</td>
</tr>
</tbody>
</table>
Table 13. (Continued)

<table>
<thead>
<tr>
<th>Percent of Jaws Filled:</th>
<th>Overall (n=45)</th>
<th>US (n=25)</th>
<th>BP (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>13.3% (6)</td>
<td>16.0% (4)</td>
<td>10.0% (2)</td>
</tr>
<tr>
<td>60%</td>
<td>2.2% (1)</td>
<td>0</td>
<td>5.0% (1)</td>
</tr>
<tr>
<td>75%</td>
<td>33.3% (15)</td>
<td>36.0% (9)</td>
<td>30.0% (6)</td>
</tr>
<tr>
<td>80%</td>
<td>2.2% (1)</td>
<td>4.0% (1)</td>
<td>0</td>
</tr>
<tr>
<td>100%</td>
<td>44.4% (20)</td>
<td>44.0% (11)</td>
<td>45% (9)</td>
</tr>
<tr>
<td>125%</td>
<td>4.4% (2)</td>
<td>0</td>
<td>100% (2)</td>
</tr>
<tr>
<td>Collagen (µg/mg)</td>
<td>62.83 (23.75)</td>
<td>63.33 (23.84)</td>
<td>62.18 (24.23)</td>
</tr>
<tr>
<td></td>
<td>10.66-113.25</td>
<td>19.23-113.25</td>
<td>10.66-103.89</td>
</tr>
<tr>
<td>Elastin (µg/mg)</td>
<td>34.524 (12.82)</td>
<td>33.84 (9.91)</td>
<td>35.411 (16.10)</td>
</tr>
<tr>
<td>C/E</td>
<td>2.20 (1.55)</td>
<td>2.10 (1.14)</td>
<td>2.33 (1.99)</td>
</tr>
<tr>
<td></td>
<td>0.33-7.55</td>
<td>0.33-6.09</td>
<td>0.82-7.55</td>
</tr>
</tbody>
</table>

*26 were randomized to US – burst pressure was not recorded for 1. Inflammation data, collagen and elastin are available for this subject, but other cystic duct characteristics were not recorded.

Hypothesis 2

Controlling for cystic duct diameter and device, C/E was not associated with ln(burst pressure) (Figure 18). In fact, an overall F test found that the predictors of C/E, duct diameter and device did not explain a significant amount of the variation in ln(burst pressure) (Multiple R-squared: 0.083, Adjusted R-squared: 0.016, Overall F-statistic: 1.25 on 3 and 41 DF, p= 0.31).
Figure 18. (a) Ln(burst pressure) by diameter and device; (b) Ln(burst pressure) by C/E ratio and device.
Table 14. Hypothesis 2 Model

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>5.737</td>
<td>0.278</td>
<td>20.615</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Group=US</td>
<td>-0.259</td>
<td>0.181</td>
<td>-1.434</td>
<td>0.159</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>-0.075</td>
<td>0.054</td>
<td>-1.388</td>
<td>0.173</td>
</tr>
<tr>
<td>C/E</td>
<td>-0.061</td>
<td>0.056</td>
<td>-1.092</td>
<td>0.281</td>
</tr>
</tbody>
</table>

Hypothesis 3

A natural log transformation of C/E was taken to meet the assumptions of multiple linear regression. Sex, age and BMI were significantly associated with ln(C/E) (Multiple R-squared: 0.22, Adjusted R-squared: 0.16, F-statistic: 3.87 on 3 and 42 DF, p=0.016). Controlling for sex and age, BMI was significantly associated with ln(C/E) (p=0.029). For each 1 unit increase in BMI, C/E decreased by 2.0% (95% CI: 0.2% to 3.7%). Controlling for sex and BMI, age was also significantly associated with ln(C/E) (p=0.010), with each one-year increase in age associated with a 1.8% reduction in C/E (95% CI: 0.5% to 3.2% decrease). Controlling for age and BMI, C/E did not significantly differ between men and women in this study (p=0.120), however relatively few men were recruited into the study, so this relationship may be difficult to discern in this dataset. One subject in the study had a BMI of 76 – removing this subject from the analysis, the point estimate for the effect of BMI on C/E remains unchanged, however the effect is no longer statistically significant (p=0.14).

Hypothesis 4

Sex, age and BMI were not significantly associated with ln(burst pressure) controlling for device (Multiple R-squared: 0.04, Adjusted R-squared: -0.06, F-statistic: 0.40 on 4 and 40 DF, p=0.81).
Table 15. Hypothesis 3 Model

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.956</td>
<td>0.412</td>
<td>4.749</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Sex = Male</td>
<td>0.399</td>
<td>0.251</td>
<td>1.589</td>
<td>0.120</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.019</td>
<td>0.007</td>
<td>-2.684</td>
<td>0.010</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.020</td>
<td>0.009</td>
<td>-2.262</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Table 16. Hypothesis 4 Model

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>5.476</td>
<td>0.438</td>
<td>12.512</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Sex = Male</td>
<td>0.049</td>
<td>0.277</td>
<td>0.176</td>
<td>0.861</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.006</td>
<td>0.007</td>
<td>-0.746</td>
<td>0.460</td>
</tr>
<tr>
<td>BMI</td>
<td>0.002</td>
<td>0.009</td>
<td>0.253</td>
<td>0.801</td>
</tr>
<tr>
<td>Group = US</td>
<td>-0.159</td>
<td>0.181</td>
<td>-0.878</td>
<td>0.385</td>
</tr>
</tbody>
</table>

Exploratory analyses

\[\text{Ln(burst pressure)}.\]

After backwards selection, the final model for \(\text{ln(burst pressure)}\) included smoking status, C/E and whether one or two bites were required to seal the duct as predictors (Multiple R-squared: 0.2, Adjusted R-squared: 0.14, F-statistic: 3.32 on 3 and 41 DF, p= 0.029). Controlling for smoking status and whether one or two bites were required, for each one-fold increase in C/E, burst pressure decreased by 12.6\% (95\% CI: 1.8\% to 22.5\% decrease, p=0.029). Controlling for smoking status and C/E, ducts that required two bites instead of one had 41.7\% lower burst pressures (95\% CI: 14.6\% to 60.2\% lower, p=0.007) (Figure 19a). Controlling for the number of bites and C/E,
smokers had 40.3% lower burst pressures than former or never smokers (95% CI: 14.6% to 60.2% lower, p=0.042) (Figure 19b).

![Figure 19a](image1.png)

**Figure 19.** (a) Ln(burst pressure) by C/E and number of bites; (b) Ln(burst pressure) by C/E and smoking status.

There were four observations in the dataset with high C/E over 5. All other subjects had C/E less than or equal to 3.08. These observations may have high influence on the results of the analysis above. Repeating the analysis without these data points, C/E is no longer included in the final model. The final model included smoking status, diabetes and whether one or two bites were required to seal the duct as predictors.
(Multiple R-squared: 0.26, Adjusted R-squared: 0.20, F-statistic: 4.25 on 3 and 37 DF, p = 0.011). Controlling for smoking status and diabetes, ducts that required two bites instead of one had 45.6% lower burst pressures (95% CI: 21.2% to 62.4% lower, p=0.002). Controlling for the number of bites and diabetes, smokers had 43.5% lower burst pressures than former or never smokers (95% CI: 9.2% to 64.8% lower, p=0.042). Controlling for smoking status and whether one or two bites were required, diabetes was not statistically significantly associated with burst pressure (p=0.062). Controlling for smoking status and whether one or two bites were required, diabetics had 55.1% higher burst pressures than others (95% CI: 2.3% lower to 146.4% higher).

Table 17. Final Ln(Burst Pressure) Model, All Subjects Included

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>5.875</td>
<td>0.227</td>
<td>25.938</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Two Bites</td>
<td>-0.540</td>
<td>0.189</td>
<td>-2.853</td>
<td>0.007</td>
</tr>
<tr>
<td>C/E</td>
<td>-0.134</td>
<td>0.059</td>
<td>-2.261</td>
<td>0.029</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>-0.515</td>
<td>0.246</td>
<td>-2.095</td>
<td>0.042</td>
</tr>
</tbody>
</table>

Table 18. Final Ln(Burst Pressure) Model, Excluding C/E > 5

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>5.610</td>
<td>0.136</td>
<td>41.118</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Two Bites</td>
<td>-0.609</td>
<td>0.183</td>
<td>-3.330</td>
<td>0.002</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>-0.571</td>
<td>0.234</td>
<td>-2.441</td>
<td>0.020</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.439</td>
<td>0.228</td>
<td>1.922</td>
<td>0.062</td>
</tr>
</tbody>
</table>
After backwards selection, the final model for ln(C/E) included age and diabetes as predictors (Multiple R-squared: 0.23, Adjusted R-squared: 0.19, F-statistic: 6.44 on 2 and 43 DF, p=0.0036). Controlling for diabetes status, age was not significantly associated with C/E (p=0.100). Each one-year increase in age was associated with a 1.0% reduction in C/E (95% CI: 2.2% reduction to 0.2% increase) (Figure 20a). Controlling for age, C/E was 47.7% lower for diabetics (95% CI: 16.7% to 67.1% lower, p=0.007) (Figure 20b).

**Collagen.**

The final model for collagen content included only diabetes as a predictor (Multiple R-squared: 0.15, Adjusted R-squared: 0.13, F-statistic: 7.66 on 1 and 44 DF, p=0.0082). On average, diabetics had 25.2 µg/mg lower collagen content (95% CI: 6.9 to 43.5 lower) (Figure 21).

**Elastin.**

The final model for elastin included age and BMI as predictors (Multiple R-squared: 0.28, Adjusted R-squared: 0.25, F-statistic: 8.55 on 2 and 43 DF, p-value: 0.00075). Controlling for BMI, for each one-year increase in age, elastin content increase by 0.44 µg/mg (95% CI: 0.20 to 0.69 increase, p=0.0006). Controlling for age, BMI was

<table>
<thead>
<tr>
<th>Table 19. Final Model Ln(C/E)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Estimate</strong></td>
</tr>
<tr>
<td>Intercept</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Diabetes</td>
</tr>
</tbody>
</table>
Figure 20. (a) Ln(C/E) by age and diabetes status; (b) box plots of Ln(C/E) by diabetes status.
Table 20. Final Collagen Model

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>66.663</td>
<td>3.550</td>
<td>18.781</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-25.190</td>
<td>9.099</td>
<td>-2.768</td>
<td>0.008</td>
</tr>
</tbody>
</table>

not significantly associated with elastin content (p=0.068). For each one-unit increase in BMI, elastin content increased by 0.32 µg/mg (95% CI: -0.02 decrease to 0.67 increase) (Figure 22).

Histology results

Sealed ducts were stained using Masson’s trichrome stain. At the seal site, cystic duct compression with full closure of the lumen was evident. There was clear evidence of collagen denaturation demonstrated by the homogenous “welded” appearance of the material in the seal (Figures 24 and 25), and by the deep purple color within the seal site.
Figure 22. Elastin content by age and obesity status.

Table 21. Final Elastin Model

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>5.303</td>
<td>7.655</td>
<td>0.693</td>
<td>0.492</td>
</tr>
<tr>
<td>Age</td>
<td>0.444</td>
<td>0.121</td>
<td>3.685</td>
<td>0.0006</td>
</tr>
<tr>
<td>BMI</td>
<td>0.322</td>
<td>0.172</td>
<td>1.873</td>
<td>0.068</td>
</tr>
</tbody>
</table>

femoral, iliac, and carotid porcine arteries stained with Masson’s trichrome (Figure 23). Elastin is much less apparent in the high collagen renal artery. Although elastin was detected in the cystic ducts biochemically using the elastin assay, there was no visual evidence of elastin fibers in the cystic duct samples (Figure 24). Similar to the porcine renal artery in Figure 23, trichrome stained cystic duct sections revealed extensive collagen with very little elastin in the cystic ducts (Figure 24).
Figure 23. Arterial collagen and elastin content is dependent on arterial type. Representative histological sections of (a) femoral, (b) iliac, (c) carotid, and (d) renal porcine arteries stained with Masson’s trichrome stain following sealing with a BP device. Masson’s trichrome stains native collagen blue, denatured collagen purple, smooth muscle red, and elastin black.

Figure 24. Representative histological sections of human cystic ducts stained with Masson’s trichrome stain following sealing with a BP device shows a well formed homogenous seal amalgam consisting of denatured collagen. Masson’s trichrome stains native collagen blue, denatured collagen purple, smooth muscle red, and elastin black.

Figure 25. Representative histological section of human cystic duct stained with H&E following sealing with a BP device shows a well formed homogenous seal amalgam, and moderate mixed inflammatory infiltrate (predominantly consisting of lymphocytes and plasma cells) in the adjacent tissue.
CHAPTER V
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

An energy-based device optimized for the cystic duct would fundamentally change the most common abdominal laparoscopic operation performed by general surgeons. The traditional use of monopolar energy for “clip and cautery” of the cystic duct and for dissection causes patients to experience pain as a result of char to the liver bed, and as a result of bile leak into the peritoneal space from perforated gallbladders [9, 16, 17, 29, 40, 62, 63, 72, 73]. In addition, bile leak from the cystic duct as a result of clip displacement is a serious, and potentially life threatening complication [26, 27]. There is an opportunity to diminish human suffering after LC by reducing postoperative pain and complications associated with monopolar cautery dissection and clip displacement. There is also an opportunity to impact health care economic metrics via reduced operating time, and a reduction in costs associated with increased length of hospital stay and with the clinical care needed to address complications.

Our primary objective with this series of experiments was to determine if US and BP energy-based surgical devices can adequately seal the cystic duct, and if the energy source impacts seal quality given duct diameter, and collagen and elastin content. We also sought to determine if the porcine model is a reasonable test model for human tissue. We used a systemic approach to answer our research question beginning with animal research, followed by a prospective cohort pilot study, and finally a randomized trial comparing US to BP energy.

Preclinical

Our objectives for the porcine study were both qualitative and quantitative. We conducted a survival study in order to determine the incidence of bile leak in a porcine model after sealing the cystic duct with BP energy. This was a qualitative measure of the
ability of the bile duct to heal after thermal insult. Our quantitative objective was to
determine the burst pressure of porcine cystic ducts sealed with BP energy both
immediately after sealing, and after seven days of healing.

The preclinical literature on the use of the BP or US energy to seal the cystic duct
is in general of poor quality, with variable methods, no a priori power calculation for
comparison experiments and inadequate reporting of data e.g. not one study reported a
95% confidence interval. As a result, there are contradictory findings across studies, and
it is difficult to draw conclusions. Matthews et al. and Hope et al. conducted nearly
identical experiments at Carolinas Medical Center eight years apart, in which they used
both BP and US energy to seal the common bile duct as opposed to the cystic duct [18,
19]. The authors suggested that the common bile duct would be a better model for
human cystic ducts given the increased size of the porcine common bile duct compared
to the porcine cystic duct. However, no diameters of either human or porcine ducts or
any other evidence supporting this assumption was reported. In these two very
influential papers, both authors reported that nearly every seal necrosed, and few pigs
survived the entire seven-day study period. Both papers concluded that using either BP
or US energy to seal the cystic duct was not feasible. During the same eight-year time
period, a number of other authors reported polar opposite results [16, 17, 26, 27, 29, 41,
62, 65, 73]. Schulze et al. published evidence in a preclinical model using an identical BP
instrument to seal porcine cystic ducts during LC with a seven-day survival period, and
all ten animals survived with no bile leak and no complications [29].

In our experiments using the porcine model, we conducted a pilot study to gain
an idea of burst pressure range in porcine cystic duct. In the survival experiments, we
burst tested the specimen side of the cystic duct immediately after LC using a BP
instrument to seal the cystic duct and artery. The animals were survived for seven days
before necropsy and burst testing the “patient” side of the seal. Our qualitative
experimental results were similar to the majority of other authors, and all the animals
survived with no complications. In addition, the burst pressures were robust, in the acute experiment, with a mean of 830.9 (SD=298.2). Burst pressures were also robust in our survival experiment, with a mean of 491.8 (SD=403.3) on the side burst immediately after excision, and a mean of 537.5 (SD=343.6) on the side excised at postoperative Day 7. The mean diameter of the porcine cystic ducts was (2.1 mm) in our experiments, and Shamiyeh et al. observed a mean cystic duct diameter of 1.8 mm in their porcine study [30]. The cystic duct diameter we observed in the human studies was somewhat larger. The mean was 4.2 mm with a range of 2.4-11mm in the pilot cohort study, and the mean was 3.0 mm with a range of 1.1-13.2 mm in the randomized controlled trial. It is unclear why our experimental results and those of other authors differed so radically from the Carolinas Medical Center experiments. Theoretically, the porcine common bile duct should not seal significantly differently than the cystic duct, however the poor results from their studies suggest otherwise. As a result of our experimental results as well as those of other authors reported in the literature in both porcine and human cystic ducts, we determined that sealing the cystic duct with BP or US energy was feasible, and were encouraged to design a pilot cohort study using human cystic duct stumps.

Pilot cohort study

Our objectives for the pilot cohort study were to determine the distribution of cystic duct burst pressures sealed with BP energy, to quantify the range of human cystic duct diameters and wall thicknesses and to understand if the previously reported predictors of vascular burst pressure such as diameter and wall thickness are also associated cystic duct burst pressure. Finally, we needed to obtain pilot burst pressure data on which to base a randomized controlled trial comparing US energy to BP energy. We also conducted an exploratory analysis of the relationship between demographic characteristics of body mass index, age, and sex to the burst pressure of the cystic duct in an effort to better understand the characteristics of the patient population in relation to
our main measure of efficacy. Based on our review of the prior clinical literature, we chose to exclude patients with acute cholecystitis from our cohort study. Many of these patients have very large, inflamed cystic ducts and we considered it unlikely that our device could successfully seal ducts of this diameter. In addition, there are other confounding factors in this population such as empyemic ducts that we did not feel we could adequately control for in our study. Prior authors who tested the devices clinically and who left the seal as the sole source of biliary-stasis also excluded acute or emergent cases from their studies.

The metric currently utilized by industry and the food and drug administration to define safe and efficacious sealing of vasculature is a mean burst pressure of at least 360 mmHg [28]. This number was first suggested by industry, and later accepted by the FDA given that it is approximately three times higher than normal systolic blood pressure of 120 mmHg. We choose to adopt a similar metric for cystic duct sealing. The normal back-pressure of the cystic duct is 10-15 mmHg [18] Based on prior criteria of three times physiologic pressure for a successful vascular seal, we (and other authors) determined that a pressure of at least 45 mmHg was necessary in the cystic duct.

In this pilot study one seal burst at 44.6 mmHg, which accounts for 2.6% of seals (95% Wilson Confidence Interval: 0.1-13.2%). While this result is encouraging, due to the sample size and small number of events in this pilot study, we cannot rule out up to 13.2% of seals bursting at or below 45 mmHg. It is worth noting that 45 mmHg is still well in excess of the 15 mmHg physiologic pressure of the cystic duct, so this may be a conservative standard in such a low-pressure system.

This cohort study is the first to describe the inner diameter, outer diameter and wall thickness of diseased cystic ducts in relation to burst pressure. Morphological characteristics such as these are often critical in assessing the potential for success or failure of energy-based sealing devices in vasculature, and current Food and Drug Administration restrictions of vessels ≤7 mm in diameter for radiofrequency sealing
devices and vessels ≤ 5 mm in diameter for ultrasonic sealing devices reflect this limitation in effectiveness [20]. Despite this, no prior studies have explored the relationship of cystic duct diameter to burst pressure, or described the diameter of the cystic duct as it relates to the severity of cholecystitis. Basic information such as this may help in clinical decision making to assess the suitability of using energy as an alternative to clips in a particular patient.

Given existing evidence of a strong relationship between vascular diameter and burst pressure, we expected to find a similar relationship between cystic duct diameter and burst pressure, but no such relationship was observed [20]. It is unclear why this is the case, however other variables such as the collagen to elastin ratio have been shown to explain a large amount of the variability in the burst pressure in vasculature, and it is possible that collagen content is even more of an important predictor of burst pressure in the cystic duct than it is in vasculature [21-23]. We were unable to find anything in the published literature describing the total collagen content or elastin content of the cystic duct so an indirect literature comparison to vasculature collagen or elastin content is not possible.

We also expected to find a relationship between wall thickness and burst pressure due to our prior work in human vasculature, and also due to the fundamental mechanism of action of BP devices [23]. BP devices work by combining precise pressure with an impedance based algorithm to regulate energy delivery. Gross examination reveals that the cystic duct has much different viscoelastic properties than vasculature. The wall is much thicker and stiffer (perhaps due to more collagen) with very little musculature relative to blood vessels. This led us to speculate that a device with higher jaw pressure would be required to achieve an adequate seal; the data did not support this hypothesis as the mean burst pressure of 288.7 mmHg was well in excess of the minimum threshold of 45mmHg. It is worth noting, that although this is a robust burst
pressure, it is far lower than the burst pressures we observed in the porcine cystic duct model.

Finally, we hypothesized that we might find an association between age, higher BMI and male sex with lower burst pressure. Previous authors have reported an increase in complications including bile duct injury associated with these demographic characteristics [74, 75]. Large registry studies have also consistently found an increase in complications in older men with high BMI [39, 74]. It is possible that these characteristics are confounded by a relationship with longstanding chronic cholecystitis, which would lead to adhesions and viscoelastic tissue and anatomical changes that may lead to more difficulty with dissection, or with application of clips to the cystic duct. We did not find an association with any of these variables and with burst pressure. Chronic cholecystitis may in fact be beneficial for BP sealing due to the increase in tissue collagen associated with chronic inflammatory processes.

**Prospective randomized controlled trial**

Building on the results of our preclinical work and the pilot cohort study, we designed a prospective randomized study to determine if BP energy was non-inferior to US energy in terms of mean or median burst pressure. As in the pilot cohort study, we limited the patient population to non-emergent and non-acute cases with chronic cholecystitis. Our objectives for the randomized controlled trial were to compare the mean burst pressure of human cystic ducts sealed with US energy or BP energy, and to evaluate the relationship between collagen and elastin content, cystic duct diameter, and burst pressure. We also conducted some exploratory analysis of patient demographic variables and their relationship to burst pressure and collagen and elastin content. Our overall goal was also to determine the best energy source with which to conduct a future clinical trial.
There is a single case series of 100 patients in which bipolar energy was used clinically to seal the cystic duct, however there are many more clinical studies with over a thousand total patients examining the use of US energy to seal the cystic duct [16, 17, 41-43, 60-65, 71]. Given the paucity of clinical evidence for BP energy, we wanted to ensure that BP energy was not inferior to US energy. We tested the primary hypothesis of non-inferiority, and ruled out clinically meaningful inferiority of the BP device compared to the US device. We expected this result, given the superior sealing ability of BP devices compared to US devices in vasculature, however no one had ever tested the hypothesis with a randomized controlled trial. The median burst pressure for the US device was 184.6 (range 52.5-700.9) and the median burst pressure for the US device was 230.6 (range of 82.7-404.8). Although these values exceed the 45-mmHg cutoff, they are substantially lower than the values we observed in the porcine model. None of the seals burst below 45 mmHg (95% CI: 0-7.9%), however with our sample size, we cannot rule out up to 7.9% of seals bursting below 45 mmHg.

**Form fits function: C/E in the cystic duct is higher than in vasculature**

In our previous work in vasculature we consistently observed a relationship of burst pressure to C/E in both a porcine model and a human cadaver model when controlling for vasculature diameter [22, 23]. We did not observe this relationship in the cystic duct. A comparison of C/E in the cystic duct to the ratios observed in human vasculature reveals that the mean C/E of the cystic duct is much higher (mean C/E of 2.198) than the mean C/E in vasculature, which ranges from a mean of 0.7 in splenic arteries to a mean of 1.6 in the femoral artery. In addition, almost all of the effect of C/E on burst pressure in vasculature occurs within the range 0.5 to 1.5, with burst pressure increasing with increasing C/E. However, once C/E reaches 1.5, additional increases in C/E do not result in significant increases in burst pressure (see change point model analysis in Appendix A). This may explain the lack of a relationship of C/E to burst
pressure in the cystic duct, as a C/E less than 1.5 is much less common in the cystic duct than in vasculature. This may also explain the lack of relationship of burst pressure to cystic duct diameter. One potential hypothesis is that there is a critical threshold of collagen needed for an adequate seal. Many blood vessels do not meet this critical threshold, however essentially all cystic ducts do. In our previous publications, we found that when one factors in collagen and elastin content, vessel functional origin, and the range of diameters examined in this study (2–5 mm), vessel diameter appears to be overcome by collagen and elastin content and vascular origin as a predictor of burst pressure. These findings are logical when one considers the physiologic functionality of renal and carotid vessels compared to the more peripheral vessels (iliacs and femorals).

A relatively low index of distensibility, correlating with a high C/E, is necessary for vessels intimately involved in maintaining homeostasis [51]. That is, excessive dilation of renal vessels would result in marked changes to the renin–angiotensin feedback loop and similarly for the role of the carotid arteries–baroreceptors in regulating systemic blood pressure. Cystic ducts likewise have a low index of distensibility due to the high collagen content and low elastin content. This alters the amount of tension in the duct wall vs. what one would observe in a structure with more distensibility. Since tension is directly proportional to the radius of the vessel or duct wall (as formulated in Laplace’s law), the vessel wall construct is largely predictable by diameter and pressure (as described by Young’s modulus of flexibility) [52]. The elastic modulus of the gallbladder is also related to physiologic functionality. In a fasting state, the gallbladder is relaxed as it and concentrates and stores bile secreted by hepatocytes. During this fasting state, the sphincter of Oddi in the duodenum is closed, and bile flows into the gallbladder through the hepatic and cystic ducts. Once an individual consumes food, the sphincter of Oddi relaxes, and production of the hormone cholecystokinin is triggered, which then stimulates the gallbladder to rhythmically contract, expelling bile into the small intestine. The strength of these contractions and the distensibility of the
cystic duct and the common bile duct as bile is forced into them are intimately related to the Young’s modulus or elasticity of these structures [76]. Some authors have suggested that the change in the mechanical function of the gall bladder and cystic duct may be related to the pathology and pain observed in acalculous cholecystitis [77, 78].

Changes in tissue elasticity are associated with pathology in a number of tissue types e.g. vascular atherosclerosis, diabetes carcinoma of the breast as well as with age and BMI, and stress responsive biomechanical remodeling [55-57]. Reduced contractility of the gallbladder also been observed in diabetic patients [79]. We hypothesized that this may also hold true for the cystic duct based on experiments by Duch et al in which porcine common bile ducts were obstructed, and then observed for changes in mechanical properties and in collagen content [58, 59]. In these experiments, obstruction for one week (a model for biliary stenosis) resulted in inflammation, thickening and stiffening of the wall, and in an increase in collagen content as measured histologically, although this is a less accurate measure of collagen content than the biochemical hydroxyproline assay.

Age and collagen alteration

In our experiments, we found that controlling for age, increasing BMI was associated with a decrease in C/E. In addition, controlling for BMI, we found that increasing age was associated with a decrease in C/E. An increase in crosslinking of collagen due to the products of the Maillard reaction has been shown to be related to age in vasculature, however why reduced collagen content in the cystic duct is associated with age and BMI is unclear [80]. One potential cause is that older individuals with high BMI may simply have more severe disease, with increased inflammation. The lack of resolution of the wound healing cycle, and the long-term inflammation observed in chronic cholecystitis may explain the decrease in collagen content that we observed in our study [55, 56]. The normal wound healing cycle is comprised of three distinct
phases: inflammation, proliferation, and maturation, and is a delicate balance of catabolic and anabolic processes. Inflammation occurs as a result of tissue injury or infection, and resolution of the inflammatory response is essential to completing the other components of the cycle and to successful healing [55]. A consequence of unchecked inflammatory response is unbalanced proteolytic activity, and up-regulation of metallioproteinases such as the collagenases MMP-1, and MMP-8. Collagen breakdown is normal during the early phase of wound healing, however there is a shift to collagen production and maturation in later phases. Chronic low-grade infection and accompanying inflammation is a hall-mark of cholecystitis and may result in the inability of the tissue to move beyond the inflammatory phase. Over time, this may result in a reduction of collagen in the diseased tissue.

**Exploratory analysis: Backwards selection models**

In an effort to better understand the relationships hypothesized in our conceptual model, and to potentially generate new hypotheses for future studies, we also conducted exploratory analyses of predictors of burst pressure, collagen, and elastin using backwards selection and all available variables as predictors.

**Predictors of burst pressure**

The final predictive model for burst pressure included smoking status, C/E and whether one or two bites was required to seal the duct as significant predictors. Controlling for C/E and smoking status, a two-bite seal burst 41.7% lower than a single bite seal, and 48.8% of the ducts required a two bite seal. This suggests that the surgeon should take extra care if two bites are required to close the duct, and may provide an argument for design of a longer jaw in a laparoscopic cholecystectomy specific instrument to accommodate larger diameter cystic ducts such as those encountered in acute cholecystitis. The presence of C/E and smoking as negative predictors of burst
pressure in the final model is more difficult to explain. The scatter plot for C/E compared to burst pressure shows four individuals with very high C/E relative to the rest of the population. These four observations exhibit high influence on the estimate for the effect of C/E on burst pressure in the model. One potential explanation for this observation is that the current jaw design may not apply enough pressure in these less compressible ducts. As C/E increases, the Young’s Modulus and hence the compressibility of the duct is altered. Jaw pressure is a key aspect of the device design, and inadequate compression of tissue results in less robust seal.

**Diabetic status predicts reduced collagen content: The Maillard reaction**

The only significant predictive variable remaining in the model for collagen content was diabetes. In our experiment, diabetes was associated with reduced collagen content, and on average diabetics had 25.2 µg/mg less collagen than non-diabetic patients. In addition, in large registry studies diabetes is consistently a predictor for poor patient outcomes after laparoscopic cholecystectomy. Interestingly, if you remove the four outliers from the model for burst pressure described above, C/E is replaced by diabetes as a significant predictor.

There is ample evidence in the literature for an association between diabetes and abnormal collagen accumulation in wound healing [79, 81-85]. The mechanism of this process may be generally described as an imbalance between catabolism and anabolism in normal wound healing. The Maillard reaction has been described extensively in relation to aging and to chronic diseases such as diabetes [80, 85]. Indeed, various effects of diabetes correspond to typical “ageing diseases,” which occur at a distinctly earlier age in patients with diabetes. All proteins are the objects of non-enzymatic extracellular glycosylation (NEG) of exposed amino groups by blood sugars, which results in increased catabolism of the proteins. Diabetics are susceptible to increased glycosylation reactions during periods of hyperglycemia, and increased activity of collagenase
protease activities (MMP-1 and MMP-8) may catabolize abnormally glycosylated collagen, leading to a decrease in collagen content in the wound, or in our case, a chronically inflamed cystic duct [83, 85].

**Age is a predictor of elastin content**

Our final exploratory model revealed age and BMI as predictive variables for elastin concentration, however only age was significant at the 0.05 level. Controlling for BMI, for each one-year increase in age, elastin content increases by 0.44 µg/mg (95% CI=0.20-0.69). There is no prior literature describing the relationship between age and elastin concentration in the cystic duct, and there have been conflicting reports on the direction of the relationship between age and elastin concentration in vasculature [80, 86, 87]. It is well known that the mechanical properties of vasculature change with age, and in particular the aorta becomes stiffer with reduced elasticity, even in the absence of atherosclerosis [56]. Cattell et al found a decrease in elastin concentration (µg elastin/mg of tissue) as a function of age in normotensive thoracic aortas from cadavers, particularly in cadavers over the age of 45 years [87]. Other groups have found a decrease in elastin concentration or no change with age in the aorta [86]. One potential cause of the increase in elastin concentration that we observed in diseased cystic ducts, may be that the amount of elastin is actually constant, while the other components of the cystic duct such as the smooth muscle are decreasing with age, resulting in an increase in the concentration of elastin in the tissue.

**Limitations**

There were some limitations in our experimental series. One important consideration is that this was a cohort of non-emergent chronic cholecystitis patients. This may limit the generalizability of our results, as patients presenting with acute cholecystitis may have necrotic or empyemic cystic duct tissue that does not heal well, or
does not seal adequately. Another important limitation in our experiments is related to external validity. The pilot study consisted of 77.5% women, and the randomized trial consisted of 88.2% women, due to the greater prevalence of cholecystitis in women. Therefore our results may not be generalizable to men with chronic cholecystitis. In addition, our studies do not provide evidence that the seal would heal human cystic ducts in vivo, although there have been numerous successful clinical studies in patients with chronic cholecystitis with US energy and one study with bipolar energy, and the results of our animal experiments suggest robust healing given that there was no decrease of burst pressure after seven days of healing compared to the half of the seal burst immediately after sealing. It is impossible to blind the surgeon as to which device is being used, so there may be unconscious performance bias in the application of the devices. We were, however, blinded to the device type while performing the collagen and elastin assays in an effort to limit detection bias. In addition, we attempted to limit allocation bias as much as possible by utilizing a random number generator for randomization a priori and individuals were blindly assigned to the US or BP study group based on this sequence as they came into clinic.

Conclusions, recommendations and future research

Conclusions

The results of this experimental series suggest that bipolar radiofrequency devices and ultrasonic devices may safely seal the cystic duct in patients with chronic cholecystitis. In addition, we have ruled out inferiority of the BP device with regard to burst pressure of sealed cystic ducts. Further, our results with regard to the impact of C/E on burst pressure are consistent with what we observed in vasculature. In vasculature, we observed a strong relationship to burst pressure controlling for diameter in arteries with C/E of less than 1.48. After that changepoint, there was no longer a
significant relationship (Appendix A). The cystic duct contains substantially more
collagen than vasculature, with a mean C/E of 2.2, and similar to our observations in
vasculature with C/E over 1.48, we did not see a relationship of C/E to burst pressure.
Finally, because the mean burst pressure of the porcine cystic ducts is substantially
higher than the mean burst pressure we observed in the human diseased cystic duct, we
do not consider it an adequate model for human chronic cholecystitis using the currently
accepted standard. In addition, given that the mean porcine cystic duct diameter is
significantly smaller than the mean diameter observed in humans with chronic
cholecystitis, more conservative criteria than three times the normal physiologic back-
pressure of the cystic duct may need to be utilized as a cutoff value for a successful seal
in the porcine model. Alternatively, larger animals or an animal model of chronic
cholecystitis to create a larger diameter duct may be of value.

**Future research**

Based on the superior dissection ability, and the demonstrated sealing capability
of the US device, our future research may include a randomized controlled clinical trial
comparing the US device for both dissection and sealing the cystic duct to clip and
cautery in chronic cholecystitis patients. This would allow us to test other potential
benefits of the US device as well, such as increased dissection speed, decreased
operating time, decreased length of stay, and reduced patient pain due to limited liver
char and bile leak.

**Recommendations**

We cannot currently recommend the US device as it is currently designed for
acute or emergent cholecystitis. The jaws on the current device are designed for 5 mm
vasculature, and as such are too short to seal these larger, inflamed cystic ducts without
multiple bites. A longer jaw would be useful in chronic cholecystitis patients as well,
given that in this study, 45% of the ducts required a two bite seal, and we observed significantly lower burst pressures with a “two bite” seal compared to a single bite seal. In acute cholecystitis, very large cystic ducts are more common, and it is possible that more than two bites could be required to seal the entire duct. This series of experiments would need to be repeated with the cystic duct specific US device in a population with acute cholecystitis to ensure that these patients can be safely treated with this alternative method.
REFERENCES


28. Covidien, 510(k) Submission. 200-2012, Food and Drug Administration, Department of Health and Human Services, United States of America: Freedom of Information Act


APPENDIX A

EFFECT OF C/E RATIO ON LN(BURST PRESSURE) IN VASCULATURE, CONTROLLING FOR VESSEL DIAMETER

Prior data from porcine vasculature was utilized to determine the change point of C/E ratio association with burst pressure controlling for diameter. Methods of data collection are detailed in Appendix E.

Methods
Scatterplots of ln(burst pressure) vs. C/E ratio suggested that the effect of C/E ratio may differ over the range of C/E ratios, with increasing C/E ratio initially resulting in increases in ln(burst pressure) and eventually plateauing for higher C/E ratios (Figure 1).

Figure 1. Ln(burst pressure) by C/E ratio with Loess fit.
A change point model, utilizing a linear spline with a single knot, was used to fit the data. A series of linear regression models were fit with ln(burst pressure) as the dependent variable and C/E ratio, max(0, C/E ratio - change point), and diameter as predictors over a range of potential change points. Change points considered were a sequence using increments of 0.01 of C/E ratios over the range observed in the data. The final estimate of the change point was determined using the profile likelihood method described by Hall et al. A final model was fit using this change point to obtain point estimates of the regression coefficients.

Bootstrapping was used to obtain a 95% confidence interval for the change point. Residuals were bootstrapped from the final model to create a total of 1,000 simulated bootstrap datasets, and the change point for each bootstrap dataset was fit as described above. A 95% confidence interval for the change point was calculated using the bias corrected and accelerated (BCa) method. BCa confidence intervals were also calculated for the slope before and after the change point. The method described by Julious (2001) was used to determine a bootstrap p value for the presence of a change of slope.

**Results**

The final model included a change point at a C/E ratio of 1.48 (Multiple R-squared: 0.3679, Adjusted R-squared: 0.3405, F-statistic: 13.39 on 3 and 69 DF, p-value: <0.0001). The final model is presented in Table 1. The model including a change point of 1.48 explained significantly more variability in ln(burst pressure) than the model without the change point (p=0.00464), and there was significant evidence for a change in the slope (bootstrap p-value: 0.005). The 95% BCa bootstrap confidence interval for the change point was (0.59, 2.00).

**Table 1. Estimates of Final Change Point Model**

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>SE</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>5.204</td>
<td>0.285</td>
<td>18.257</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C/E Ratio</td>
<td>1.101</td>
<td>0.209</td>
<td>5.281</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Max(C/E Ratio - 1.48, 0)</td>
<td>-1.092</td>
<td>0.373</td>
<td>-2.926</td>
<td>0.0046</td>
</tr>
<tr>
<td>Diameter</td>
<td>-0.045</td>
<td>0.077</td>
<td>-0.582</td>
<td>0.5623</td>
</tr>
</tbody>
</table>

**CE and Ln(bp) Change Point Models**

```r
# Read in Data
dat<-read.csv('/Users/camillemoore/Documents/kim_ce_vasculature/Sheet1-Table 1.csv')
dat<-dat[is.na(dat$bp)==F,]
```
dat<-dat[dat$bp != 0,]
(dat<-dat[is.na(dat$ce_ratio)==F,])
t1<-dat$ce_ratio
diam<-dat$vessel_size
y<-log(dat$bp)
dat$logbp<-y

# Search for Change Point
aic<-NULL
for (i in 1:150){
  cp<-0.5+i/100
  t2<-ifelse(t1>cp, t1-cp, 0)
  warning<-0
  tryCatch(cp.model<-lm(y~t1+t2+diam),
  warning = function(warn){warning <<-1})
  K <- matrix(c(0, 1, 1, 0), 1)
  t <- glht(cp.model, linfct = K)
  aic<-rbind(aic,
    data.frame(changepoint=cp,
    ll=(logLik(cp.model)),
    int=cp.model$coefficients[1],
    beta1=cp.model$coefficients[2],
    beta2=cp.model$coefficients[3],
    beta3=cp.model$coefficients[4],
    slope2=cp.model$coefficients[2]+cp.model$coefficients[3],
    se1=(summary(cp.model)$coefficients[1,2],
  )}
se2=(summary(cp.model))$coefficients[2,2],
se3=(summary(cp.model))$coefficients[3,2],
se4=(summary(cp.model))$coefficients[4,2],

seslope2=(summary(t)$test$sigma)

)

}

# Determine the CP
plot(aic$change_point, aic$ll, type='l', xlab='Change Point (months)', ylab='Log Likelihood')

cp<tail(aic[order(aic$ll),],1)$change_point# maximum is 1.48

# Fit Final CP Model
t2<-ifelse(t1>cp, t1-cp, 0)

summary(cp.model<-lm(y~t1+t2+diam))

# Get Bootstrap CI's for the Change Point and Coefficients

### Bootstrap Residuals

cp.resid<-resid(cp.model) # Residuals from CP Model
cp.fitted<-fitted(cp.model) # Fitted Values from CP Model

lm.fitted<-fitted(lm(y~t1+diam)) # Fitted Values from Model with no CP (for calc of p value for a change)

cp.data<-data.frame(y=y[is.na(diam)==F], t1=t1[is.na(diam)==F], diam=diam[is.na(diam)==F], cp.resid, cp.fitted, lm.fitted)

# Bootstrap Function
cp.fun <- function(dat, inds) {
  aic <- NULL
  for (zz in 1:150) {
    cp <- -0.5 + zz / 100
    t2 <- ifelse(cp.data$t1 > cp, cp.data$t1 - cp, 0)
    warning <- 0
    tryCatch(lm.b <- lm(cp.fitted + cp.resid[inds] ~ t1 + t2 + diam, data = dat),
             warning = function(warn) {warning <<- 1})
    aic <- rbind(aic, data.frame(changepoint = cp,
                                  ll = logLik(lm.b),
                                  int = lm.b$coefficients[1],
                                  beta1 = lm.b$coefficients[2],
                                  beta2 = lm.b$coefficients[3],
                                  beta3 = lm.b$coefficients[4],
                                  slope2 = lm.b$coefficients[2] + lm.b$coefficients[3])
  }
  c(tail(aic[order(aic$ll),], 1)$changepoint,
    tail(aic[order(aic$ll),], 1)$beta1, tail(aic[order(aic$ll),], 1)$beta2,
    tail(aic[order(aic$ll),], 1)$slope2, tail(aic[order(aic$ll),], 1)$int,
    tail(aic[order(aic$ll),], 1)$beta3)
# Perform Bootstrap and Calc CI's

\[
\text{cp.boot} \leftarrow \text{boot}(\text{cp.data}, \text{cp.fun}, R = 999)
\]

\[
\text{boot.ci(cp.boot, type='all', index=1)}
\]

\[
\text{boot.ci(cp.boot, type='all', index=2)}
\]

\[
\text{boot.ci(cp.boot, type='all', index=3)}
\]

\[
\text{boot.ci(cp.boot, type='all', index=4)}
\]

# Find Bootstrap P for a change in Slope

# Function to Calculate the F statistic for the Change Point under the Null of no CP

\[
\text{lm.fun}\leftarrow\text{function(dat, inds)}\
\quad \text{t2}\leftarrow\text{ifelse(cp.data}$t1>1.48, \text{cp.data}$t1-1.48, 0)\
\quad \text{lm.b}\leftarrow\text{lm(lm.fitted+cp.resid[inds]$t1+t2+diam, data=dat)}\
\quad \text{lm.1}\leftarrow\text{lm(lm.fitted+cp.resid[inds]$t1+diam, data=dat)}\
\quad \text{lm.1}\leftarrow\text{lm(lm.fitted+cp.resid[inds]$t1+diam, data=dat)}\
\quad f\leftarrow\text{68*0.5*(anova(lm.1)[3,2]-anova(lm.b)[4,2])}/\text{anova(lm.b)[4,2]}\
\quad f
\]

# Perform Bootstrap

\[
\text{lm.boot}\leftarrow\text{boot}(\text{cp.data}, \text{lm.fun}, R = 1000)
\]

# Calculate p value

\[
\text{length(lm.boot$t[(lm.boot$t>}(68*0.5*(\text{anova(lm(y$t1+diam))[3,2]-anova(cp.model)[4,2])}/\text{anova(cp.model)[4,2]})]}/1000
\]
Cystic Duct Analyses

#Load Data

pilot<-read.csv('/home/camille/Desktop/UCHSC_pilot.csv')

dat<-read.csv('/home/camille/Desktop/cystic_duct_final.csv')

ce<-read.csv('/home/camille/Dropbox/final_ce_results.csv')

colnames(ce)[1]<-'patid'

dat<-merge(dat, ce)

#Pilot Data Analysis

pilot$logbp<-log(pilot$bp)

pilot$thickness<-(pilot$diam-pilot$inner_diam)/2

summary(pilot)

#plots of BP distribution

hist(pilot$bp)

hist(pilot$logbp)

boxplot(pilot$logbp)

boxplot(pilot$bp)

#Hypothesis 1: BP is associated with inner or outer diam

hyp1<-lm(bp~diam + inner_diam, data=pilot)

summary(hyp1)
plot(hyp1)

#Hypothesis 2: BP is associated with wall thickness
hyp2 <- lm(bp ~ thickness, data = pilot)
summary(hyp2)
plot(hyp2)

#Hypothesis 3: BP is associated with BMI, sex, and age
hyp3 <- lm(bp ~ bmi + sex + age, data = pilot)
summary(hyp3)
plot(hyp3)

# RCT Data analysis

dat$thickness <- (dat$diam_o - dat$diam_i)/2
dat$smoke <- ifelse(dat$smoking == 1, 1, 0)
dat$logbp <- log(dat$bp)

summary(dat)
by(dat, dat$group, summary)

# Plots of BP distribution
boxplot(logbp ~ group, data = dat)
hist(dat[dat$group == 'US', ]$bp)
hist(dat[dat$group == 'LS', ]$bp)
# Hypothesis 1: Test for Non-Inferiority

```r
t.test(logbp~group, data=dat)
```

# Hypothesis 2: Controlling for diam and group, CE is associated with BP

```r
rct2<-lm(logbp~group+diam_o+ce_ratio, data=dat)
summary(rct2)
plot(rct2)
```

# Hypothesis 3: Male sex, increasing age, and higher BMI are associated with lower C/E ratio.

```r
rct3<-lm(ce_ratio~bmi+sex+age, data=dat)
summary(rct3)
plot(rct3) # take log transformation of CE
```

```r
dat$logce<-log(dat$ce_ratio)
```

```r
rct3<-lm(logce~bmi+sex+age, data=dat)
summary(rct3)
plot(rct3)
```

# Fit model without subject with BMI of 76

```r
summary(lm(logce~bmi+sex+age, data=dat[bmi<75,]))
```

# Hypothesis 4: Male sex, increasing age, and higher BMI are associated with lower BP.

```r
rct4<-lm(logbp~bmi+sex+age+group, data=dat)
summary(rct4)
```
Plot(rct4)

# Exploratory Analyses

dat$pjd<-ifelse(dat$percent_jaw<100,0,1)

# Burst Pressure - backwards selection

summary(lm(logbp~pjd+ce_ratio+smoke+age+sex+diam_o+BMI+group+diabetes, data=dat))
summary(lm(logbp~pjd+ce_ratio+smoke+sex+diam_o+BMI+group+diabetes, data=dat))
summary(lm(logbp~pjd+ce_ratio+smoke+sex+diam_o+group+diabetes, data=dat))
summary(lm(logbp~pjd+ce_ratio+smoke+group+diabetes, data=dat))
summary(lm(logbp~pjd+ce_ratio+smoke, data=dat))
plot(lm(logbp~pjd+ce_ratio+smoke, data=dat))

# Excluding CE ratios >5

summary(lm(logbp~pjd+ce_ratio+smoke+age+sex+diam_o+BMI+group+diabetes, data=dat[dat$ce_ratio<5,]))
summary(lm(logbp~pjd+smoke+age+sex+diam_o+BMI+group+diabetes, data=dat[dat$ce_ratio<5,]))
summary(lm(logbp~pjd+smoke+sex+diam_o+BMI+group+diabetes, data=dat[dat$ce_ratio<5,]))
summary(lm(logbp~pjd+smoke+sex+diam_o+group+diabetes, data=dat[dat$ce_ratio<5,]))
summary(lm(logbp~pjd+smoke+sex+group+diabetes, data=dat[dat$ce_ratio<5,]))
summary(lm(logbp~pjd+smoke+group+diabetes, data=dat[dat$ce_ratio<5,]))
summary(lm(logbp~pjd+smoke+diabetes, data=dat[dat$ce_ratio<5,])))

#Collagen
summary(lm(collagen~smoke+age+sex+diam_o+BMI+diabetes, data=dat))
summary(lm(collagen~smoke+sex+diam_o+BMI+diabetes, data=dat))
summary(lm(collagen~smoke+sex+BMI+diabetes, data=dat))
summary(lm(collagen~smoke+sex+diabetes, data=dat))
summary(lm(collagen~sex+diabetes, data=dat))
summary(lm(collagen~diabetes, data=dat))

#Elastin
summary(lm(elastin~smoke+age+sex+diam_o+BMI+diabetes, data=dat))
summary(lm(elastin~smoke+age+sex+BMI+diabetes, data=dat))
summary(lm(elastin~smoke+age+sex+BMI, data=dat))
summary(lm(elastin~age+sex+BMI, data=dat))
summary(lm(elastin~age+BMI, data=dat))

#CE Ratio
summary(lm(logce~smoke+age+sex+diam_o+BMI+diabetes, data=dat))
summary(lm(logce~smoke+age+sex+BMI+diabetes, data=dat))
summary(lm(logce~age+sex+BMI+diabetes, data=dat))
summary(lm(logce~age+sex+diabetes, data=dat))
summary(lm(logce~age+diabetes, data=dat))

#Plots
#BP vs. CE Ratio
plot(x=dat$ce_ratio, y=dat$bp, type='p', pch=1, xlab='C/E', ylab='Burst Pressure (mmHg)')
points(x=dat[dat$group=='US',]$ce_ratio, y=dat[dat$group=='US',]$bp, pch=16)
legend('topright', c('BP', 'US'), pch=c(1,16))

# log BP vs. CE
plot(x=dat$ce_ratio, y=dat$logbp, type='p', pch=1, xlab='C/E', ylab='Ln Burst Pressure (mmHg)')
points(x=dat[dat$group=='US',]$ce_ratio, y=dat[dat$group=='US',]$logbp, pch=16)
legend('topright', c('BP', 'US'), pch=c(1,16))

# Burst Pressure vs. Diameter
plot(x=dat$diam_o, y=dat$bp, type='p', pch=1, main='Burst Pressure by Diameter and Device', xlab='Diameter (mm)', ylab='Burst Pressure (mmHg)')
points(x=dat[dat$group=='US',]$diam_o, y=dat[dat$group=='US',]$bp, pch=16)
legend('topright', c('LS', 'US'), pch=c(1,16))

plot(x=dat$diam_o, y=dat$logbp, type='p', pch=1, xlab='Diameter (mm)', ylab='Ln Burst Pressure (mmHg)')
points(x=dat[dat$group=='US',]$diam_o, y=dat[dat$group=='US',]$logbp, pch=16)
legend('topright', c('BP', 'US'), pch=c(1,16))

# Burst Pressure vs. Percentage of Jaws filled
plot(x=dat$percent_jaw, y=dat$bp, type='p', pch=1, main='Burst Pressure by Percentage of Jaws Filled and Device', xlab='Percentage of Jaws Filled', ylab='Burst Pressure (mmHg)')
points(x=dat[dat$group=='US',]$percent_jaw, y=dat[dat$group=='US',]$bp, pch=16)
legend('topright', c('LS', 'US'), pch=c(1,16))
plot(x=dat$percent_jaw, y=dat$logbp, type='p', pch=1, main='Ln(Burst Pressure) by Percentage of Jaws Filled and Device', xlab='Percentage of Jaws Filled', ylab='Ln Burst Pressure (mmHg)')

points(x=dat[dat$group=='US',]$percent_jaw, y=dat[dat$group=='US',]$logbp, pch=16)
legend('topright', c('LS', 'US'), pch=c(1,16))

#Burst Pressure vs. smoking
boxplot(bp~smoke, data=dat, names=c('Never/Former', "Current Smoker"), ylab='Burst Pressure (mmHg)')
boxplot(logbp~smoke, data=dat, names=c('Never/Former', "Current Smoker"), ylab='Burst Pressure (mmHg)')

plot(x=dat$ce_ratio, y=dat$logbp, type='p', pch=1, main='Ln(Burst Pressure) by CE Ratio and Smoking Status', xlab='CE Ratio', ylab='Ln Burst Pressure (mmHg)')
points(x=dat[dat$smoke==1,]$ce_ratio, y=dat[dat$smoke==1,]$logbp, pch=16)
legend('topright', c('Never/Former', 'Current'), pch=c(1,16))

#Burst Pressure by CE and # of Bites
plot(x=dat$ce_ratio, y=dat$bp, type='p', pch=1, main='Burst Pressure by CE Ratio and Number of Bites', xlab='CE Ratio', ylab='Burst Pressure (mmHg)')
points(x=dat[dat$pjd==1,]$ce_ratio, y=dat[dat$pjd==1,]$bp, pch=16)
legend('topright', c('1 bite', '2 bites'), pch=c(1,16))

plot(x=dat$ce_ratio, y=dat$logbp, type='p', pch=1, main='Ln Burst Pressure by CE Ratio and Number of Bites', xlab='CE Ratio', ylab='Ln Burst Pressure (mmHg)')
points(x=dat[dat$pjd==1,]$ce_ratio, y=dat[dat$pjd==1,]$logbp, pch=16)
legend('topright', c('1 bite', '2 bites'), pch=c(1,16))

boxplot(dat$bp~dat$pjd, names=c('1 bite', '2 bites'), ylab='Burst Pressure (mmHg)')
boxplot(log(dat$bp)~dat$pjd, names=c('1 bite', '2 bites'), ylab='Ln Burst Pressure (mmHg)')

boxplot(log(dat$bp)~dat$pjd*dat$smoke, ylab='Ln Burst Pressure (mmHg)')

# Elastin by Age
dat$obese<-ifelse(dat$BMI>30, 1, 0)
plot(dat$age, dat$elastin, xlab='Age (years)', ylab='Elastin Content (ug/mg)', pch=1)
points(dat[dat$obese==1,]$age, dat[dat$obese==1,]$elastin, pch=16)
legend('topright', c('Not Obese', 'Obese'), pch=c(1,16))

# Elastin by BMI
plot(dat$BMI, dat$elastin, xlab='Age (years)', ylab='Elastin Content (ug/mg)', pch=1)
boxplot(elastin~obese, data=dat, names=c('Not Obese', 'Obese'), ylab='Elastin Content (ug/mg)')

# Collagen by Diabetes
boxplot(collagen~diabetes, data=dat, names=c('Non-Diabetic', 'Diabetic'), ylab='Collagen Content (ug/mg)')

# Ce ratio by age and diabetes
boxplot(ce_ratio~diabetes, data=dat, names=c('Non-Diabetic', 'Diabetic'), ylab='CE Ratio')
boxplot(log(dat$ce_ratio)~diabetes, data=dat, names=c('Non-Diabetic', 'Diabetic'), ylab='Ln(CE Ratio)')

plot(dat$age, dat$ce_ratio, xlab='Age (years)', ylab='CE Ratio', pch=1)
points(dat[dat$diabetes==1,]$age, dat[dat$diabetes==1,]$ce_ratio, pch=16)
legend('topright', c('Non-Diabetic', 'Diabetic'), pch=c(1,16))
Given a change point of 1.48, for C/E ratios less than 1.48, each 1 unit increase in C/E ratio resulted in a 1.1 unit increase in \( \ln(burst\ pressure) \) (95% CI: -0.422 to 0.441) increase in \( \ln(burst\ pressure) \) or a 201% increase in burst pressure (95% CI: 98% to 356% increase in burst pressure). Above 1.48, the effect of changes in C/E ratios on \( \ln(burst\ pressure) \) was significantly reduced (\( p=0.005 \)). Above 1.48, there was no longer a significant effect of C/E ratio on \( \ln(burst\ pressure) \) (\( p=0.97 \)), with each 1 unit increase in C/E ratio resulting in an estimated 0.0091 (95% CI: 34.5% reduction to 55.4% increase) (Figure 2).

Figure 2. \( \ln(burst\ pressure) \) by C/E ratio with final model.

The standard errors and confidence intervals for the effect of C/E ratio on burst pressure presented in Table 1 depend on the change point being a fixed, known value of 1.48. 95% BCa confidence intervals were also calculated for the effect of C/E ratio before and after an unknown change point (Table 2).
Table 2. 95% BCa Bootstrap Confidence Intervals for the Effect of C/E Ratio on Ln(Burst Pressure) before and After the Change Point

<table>
<thead>
<tr>
<th></th>
<th>Change in ln(Burst Pressure)</th>
<th>Percent Change in Burst Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>95% BCa Confidence Interval</td>
</tr>
<tr>
<td>C/E Ratio Before Change</td>
<td>1.101</td>
<td>0.662</td>
</tr>
<tr>
<td>Change Point</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/E Ratio after CP</td>
<td>0.009</td>
<td>-0.721</td>
</tr>
<tr>
<td>Difference</td>
<td>-1.092</td>
<td>-1.753</td>
</tr>
</tbody>
</table>


APPENDIX B

CYSTIC DUCT MODEL WITH COLLAGEN AND ELASTIN, CONTROLLING FOR GROUP AND DIAMETER

Multiple linear regression was used to model ln(burst pressure). Predictors included group, diameter, elastin content and collagen content. These predictors failed to explain a significant proportion of the variation in ln(burst pressure) (Multiple R-squared: 0.07073, Adjusted R-squared: -0.02219, F-statistic: 0.7612 on 4 and 40 DF, p-value: 0.5568).

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>SE</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>5.554554</td>
<td>0.389665</td>
<td>14.255</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Group=US</td>
<td>-0.235636</td>
<td>0.183246</td>
<td>-1.286</td>
<td>0.206</td>
</tr>
<tr>
<td>Diameter</td>
<td>-0.072738</td>
<td>0.055458</td>
<td>-1.312</td>
<td>0.197</td>
</tr>
<tr>
<td>Elastin</td>
<td>0.004105</td>
<td>0.006979</td>
<td>0.588</td>
<td>0.560</td>
</tr>
<tr>
<td>Collagen</td>
<td>-0.001789</td>
<td>0.003695</td>
<td>-0.484</td>
<td>0.631</td>
</tr>
</tbody>
</table>
APPENDIX C

THE IMPACT OF ATHEROSCLEROSIS AND VASCULAR COLLAGEN ON ENERGY-BASED VESSEL SEALING

The impact of atherosclerosis and vascular collagen on energy-based vessel sealing

Kimberly Martin, MSCS,a,b,* Kimberly Krugman, MS,a Cassandra Latimer, MS,a and Camille Moore, MSa

aUniversity of Colorado Anschutz Medical Campus, Aurora, Colorado
bCovidien Energy Based Devices, Boulder, Colorado

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ABSTRACT

Background: Bipolar energy ligation of vessels in surgery is common. Although rare, serious failures occur. Atherosclerosis may contribute to seal failures by altering vascular compressibility and collagen content; however, no data exist.

Materials and methods: Femoral and iliac arteries of six Yucatan swine with an identified genetic locus predisposing them to atherosclerosis were denuded with a Fogarty catheter. Animals were fed a high-fat diet for 28 wk. A Yorkshire pig was used as a normal control and fed a standard diet. At 28 wk, arteries were measured for their diameters, sealed, and divided in vivo with ligature. The sealed artery sections were excised and subjected to burst pressure testing. Half of the seal distal to the aorta was kept intact for histology and collagen and elastin quantification. A multiple linear regression model was used to assess variables contributing to burst pressure. Covariates included were vessel diameter, degree of atherosclerosis, and collagen content.

Results: Experimental animals were hypercholesterolemic. Atherosclerosis occurred in 90% of seals in induced animals, with severe atherosclerosis in 62% of seals. There was site-selective deposition of atherosclerotic plaques in larger diameter iliac vessels. A model including collagen and size best predicted burst pressure. Every 10 U increase in collagen resulted in 15% increase in burst pressure (95% confidence interval – 0.2%–22%, P = 0.047, R2 = 0.39). Atherosclerosis was unrelated to burst pressure controlling for collagen and size.

Conclusions: Collagen and size provide the best model fit for predicting burst pressure. Quantitative research in human vasculature is warranted to better understand the influence of atherosclerosis and collagen content on seal failures.

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1. Introduction

The use of energy-based vessel sealing devices (EBSDs) in the operating room for minimally invasive surgery has become commonplace. There are at least six companies with Food and Drug Administration (FDA) clearance to market EBSDs to date [1]. More than 7 million Americans have symptomatic atherosclerotic artery disease, with many of the patients who require surgical procedures falling into the highest risk demographically for development of atherosclerotic lesions [2]. This suggests that EBSDs are used on atherosclerotic vasculature. Despite this, no testing on atherosclerotic vasculature has ever been performed.

Although these devices are generally reliable, failures do occur. Given the large number of devices on the market, a moderate improvement in performance translates into
clinically meaningful reduction in complications and associated health care costs over the population as a whole. The potential for improvement in vessel sealing, a reduced rate of hemorrhage, and translation into sealing other tissues [3-12] exists through a better understanding of the basic mechanism of sealing tissues [13]. There are a substantial number of unexplained vessel seal failures resulting in hemorrhage, leak, reoperation, conversions, and other significant clinical interventions documented in the surgical literature and the FDA Manufacturer and User Facility Device Experience database [14,15], however, an accurate count is difficult to establish because the database is limited to 500 reports/y. Given that both of these forums rely on self-reporting by industry and surgeons and the reports are biased to more severe outcomes, it is likely that the actual incidence of seal failure (particularly intraoperative seal failure) is underestimated. Surgical observation and empirical experience of intraoperative seal failures suggest that one contributing factor may be the morphometric and compositional changes that occur in commonly sealed vasculature with the development of advanced atherosclerosis [15]. However, no quantitative evidence in the form of the standard metric of vessel burst pressure exists to date for any human vasculature or diseased vasculature (human or animal model) as all testing for FDA clearance is done on excised, healthy, porcine renal arteries [16]. The objective of this study is to examine the variables influencing the seal quality as measured by the vessel burst pressure of a common EBSB across a range of healthy and diseased vasculature in an atherosclerotic porcine model. Our hypotheses are as follows: (1) Controlling for vessel diameter, more severe atherosclerosis is associated with decreased burst pressure; (2) Controlling for vessel diameter, increases in collagen content are associated with higher burst pressure; and (3) Collagen content mediates the relationship between atherosclerosis and burst pressure. Atherosclerosis causes a reduction in collagen content that in turn reduces burst pressure.

2. Materials and methods

2.1. Surgical procedures and experimental model

All studies and procedures were approved by the Institutional Animal Care and Use Committee and performed in compliance with the Guide for the Care and Use of Laboratory Animals. Six juvenile, female, Yucatán “miniature swine” (initially 15–20 kg) were obtained from the Sinclair Bio Resources (Columbia, MO) colony, and one Yorkshire pig of similar age was used as a non-atherosclerotic control animal. We chose a single normal control animal on the premise that the experimental animals would demonstrate a range of atherosclerosis severity from very mild to very severe. Given an expected yield of 15–20 seals per animal, a single animal with zero atherosclerosis should suffice.

Animals were acclimated for 7–10 d before use. Pigs have been shown to be an excellent model for atherosclerosis as they are similar to humans in terms of cardiovascular anatomy and serum lipoprotein profile [17]. This line of Yucatan miniature swine was chosen because they are bred selectively to maintain an identified genetic locus, which leads to a predisposition to atherosclerosis, and also their smaller size relative to standard swine allows for the long-term high-fat diet needed to develop high-grade plaques without a prohibitive increase in weight and size. Atherosclerosis is a disease of age, and although pigs will naturally develop atherosclerotic lesions given enough time, a number of authors have demonstrated that high-grade plaques can be produced in months as opposed to years by causing slight endovascular injury via abrasion and denuding of the vasculature endothelium before subjecting the animals to a high-fat diet [17,18]. We followed the method of Isner and Gal [17] in our experiments as described in the following.

Before the denuding procedure, the experimental animals were fed a high-fat diet consisting of Purina pig chow supplemented with 4% of cholesterol, 8% of peanut oil, 8% of melted lard, 8% of whey, 40% salt, and 10% ergocalciferol (Winthrop-Breon, New York, NY) for 2 wk. For the initial surgery, the pigs were placed in a supine position and monitored with pulse oximetry. General anesthesia was induced with 4.4 mg/kg of Telazol, 0.04 mg/kg of atropine, and 1.5 mg/kg of xylazine (given intramuscularly) and maintained with isoflurane inhalational anesthetic after endotracheal intubation. The femoral and iliac arteries were chosen because of the ease of access for catheterization and their size (2–7 mm in diameter). To denude the femoral and iliac arteries, the distal femoral arteries on both sides of the animals were exposed via a standard minimally invasive cut down, with care taken to preserve the femoral artery, nerve, and vein. A 20- to 22-gauge IV needle was inserted superficially into the artery. A 40-cm long guide wire was threaded through the needle, and the needle was subsequently removed. An angioplasty catheter introducer was inserted over the wire into the femoral artery, followed by a second, larger 4F introducer, and a bolus of intravenous heparin (500 U/kg of body weight) was administered. Finally, a 40-cm long 3.5F Fogarty balloon catheter was advanced through the introducer into the femoral and iliac artery, to the terminal aorta. Once the catheter was adequately inserted, the balloon was inflated with 0.9 mL of saline according to the manufacturer's instructions for use and then pulled slowly back while deflating to denude the endothelium, then reinserted, and reinflated. The denuding process was performed for a total of five times. The animals were recovered and fed the same high-fat diet for at least 28 wk until necropsy. The control animal was fed a normal diet and did not undergo catheterization.

At approximately 28 wk, the animals were subjected to the same general anesthesia protocol described previously. Before full dissection and exposure of the iliac and femoral vessels, a midline laparotomy incision was made, and contrast media were injected into the aorta. Fluoroscopy was performed to visually assess the degree of thrombosis and calcification in the arteries to place the seals adjacent to or directly on atherosclerotic lesions. To access the arteries of interest, the rectus abdominis muscles were dissected off of their origin at the pubis using Bovie electrocautery. Once exposure was gained, the retroperitoneum was incised, and the iliac vessels were exposed from the aortic bifurcation to the inguinal ligament. In all arterial exposures, great care was taken to leave the periarterial tissue intact to prevent arteries from
retracting once they were divided. The midline incision was then extended over the inguinal region, and the inguinal ligament was divided, exposing the femoral vasculature.

Once adequate exposure was obtained, the arteries were measured for their diameters in vivo with digital calipers, then sealed, and divided in vivo with the LigaSure Atlas (Coviden, Energy Based Devices Boulder, CO) moving caudal to cranial from the peripheral femoralis, to the iliacs, to the terminal aortic branch, to ensure that blood flow was maintained during each seal. The sealed artery sections were then excised with the side of the seal proximal to the aorta (the patient side or the side that would be left behind in surgery) subjected to burst pressure testing as previously described by multiple authors [19–24]. The half of the seal distal to the aorta was kept intact for histologic analysis and collagen and elastin quantification.

2.2. Histologic and pathologic analysis

Immediately after burst testing (in the case of the proximal seal half) and excision (in the case of the distal seal half), the arteries were placed into neutral buffered formalin for histologic staining and collagen and elastin quantification. Single cross section and a longitudinal section to include the seal were taken from the distal segments of the sealed arteries, and up to three cross sections were taken from the proximal segment of the sealed arteries (after burst test) to determine the mean percentage of occlusion in that section. Arteries were processed routinely to microslides and stained with hematoxylin and eosin and modified trichrome stains. The slides were evaluated by an independent veterinary pathologist for atherosclerosis grade using the Stary system developed by the Committee on Vascular Lesions of the Council on Arteriosclerosis and American Heart Association [25] (Table 1) and for seal quality. In addition, the percentage of the circumference of the intima (in cross section) affected by atherosclerosis was estimated along with mineralization (calcification) and alteration of the tunics media.

2.3. Collagen and elastin quantification

After histologic staining, remaining paraffin-embedded tissue was deparaffinized and rehydrated, taking care to ensure that the arterial specimen was matched to the burst pressure and diameter measurements and divided equally for collagen and elastin quantification. The hydroxyproline assay for collagen quantification was based on the method described by Edwards and Obrien [26] and modified by Reddy and Enwemeka [27] and was performed as described in detail previously [13]. Elastin quantification was also performed as described in detail previously [13].

2.4. Morphometric luminal occlusion analysis

Whole slide digital images were captured on an Aperio ScanScope digital slide scanner (Aperio, Vista, CA). The ScanScope software interface (Aperio) allowed the user to scan up to five slides at one time while having the option of selecting the area of the slide to be scanned, selecting markers for even background shading, and adding focus points to optimize image sharpness. The total lumen area was calculated using the hematoxylin and eosin-stained slides. The analysis was performed by Image Pro Plus version 6.0 with a custom subroutine to calculate the area of the lumen.

2.5. Data analysis and statistical methods

Pearson correlations between burst pressure, collagen content, vessel diameter, and percent occlusion were calculated to gain a preliminary understanding of univariate relationships. The degree of atherosclerosis was measured by maximum percent luminal occlusion. A multiple linear regression framework was then used to test each hypothesis of interest. A natural log transformation of burst pressure was made to satisfy the assumptions of linear models. Model fit was assessed with scatter and quantile—quantile plots of studentized deleted residuals. Studentized deleted residuals, leverage, and influence statistics were used to evaluate the impact of outliers on model fit, and sensitivity analyses were performed to identify and determine the impact of outliers on analyses. Mediation effects were assessed according to the method of Baron and Kenny [28]. The type I error rate was set at 0.05 for all statistical tests. To test our hypothesis that controlling for vessel diameter, atherosclerosis is associated with decrease in burst pressure, a linear regression model was fit, regressing maximum luminal occlusion and vessel diameter on burst pressure. The parameter estimate for the effect of luminal occlusion was evaluated to determine the association between luminal occlusion and burst pressure, controlling for diameter. In addition, a univariate model regressing luminal occlusion on size was also created to assess the relationship between these variables.

Similarly, to test our second hypothesis that controlling for vessel size, collagen content is associated with burst pressure, a linear model regressing collagen content and vessel diameter on burst pressure was created. The parameter estimate for the effect of collagen content was evaluated to determine the association between collagen content and burst pressure, controlling for vessel diameter. A univariate model regressing collagen content on vessel diameter was also created to assess the relationship between these two variables.

---

**Table 1 – Stary/American Heart Association scale of atherosclerosis.**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Initial lesion—isolated macrophage foam cells</td>
</tr>
<tr>
<td>II</td>
<td>Fatty streak lesion—mainly intracellular lipid accumulation</td>
</tr>
<tr>
<td>III</td>
<td>Intermediate lesion—type 2 changes with small extracellular lipid pools</td>
</tr>
<tr>
<td>IV</td>
<td>Atheroma lesion—type 2 changes and core of extracellular lipid</td>
</tr>
<tr>
<td>V</td>
<td>Fibroatheroma lesion—lipid core and fibrotic layer, multiple lipid cores and fibrotic layers, mainly calcific, or mainly fibrotic</td>
</tr>
<tr>
<td>VI</td>
<td>Complicated lesion—surface defect, hematoma, hemorrhage, and thrombus</td>
</tr>
</tbody>
</table>
If luminal occlusion was found to be significantly associated with burst pressure when controlling for vessel diameter and associated with collagen content, then the mediational effect of collagen would be tested. To assess mediation, size, luminal occlusion, and collagen content would be regressed on burst pressure, and parameter estimates for the effect of luminal occlusion would be compared between the models including and excluding collagen content. Percent mediation would be calculated as the percent change in this parameter estimate.

3. Results

3.1. Extent of atherosclerosis

At 28 wk, extensive atherosclerosis developed in the arteries of animals subjected to atherogenic diet with arterial denuding. The mean plasma lipid protein levels of the experimental and the control animals were well in excess of normal, (≈73 mg/dL) and are shown in Figure 1 by the month of the experiment. From 69 sealed samples, 159 arterial sections were examined morphometrically. The percentages of examined arteries with disease by location or vessel type and grade of atherosclerosis are listed in Table 2. Representative photomicrographs are shown in Figure 2. Overall, atherosclerosis was observed in 80% of examined vessels in induced animals, and severe atherosclerosis (grade V or VI fibroatheroma) was observed in 62%. Of the total, 97% of the grade V–rated and 100% of the grade VI–rated arterial sections had medial alteration. Of the total, 86% of the grade V and 75% of the grade VI had mineralization. The mean percentage of luminal occlusion by morphometry was 30% (±0.025). Of note, there was some site-selective susceptibility to atherosclerosis in the iliac vessels, which are also larger in diameter than the femoral vessels. Experimental animal 4 died before the scheduled experimental end date, at approximately 27 wk. A necropsy was performed, and samples of the heart and lungs of the animal were sent to Colorado State University Veterinary Diagnostic Lab to be evaluated by a veterinary pathologist. The pathology report described severe atherosclerotic changes in the cardiac vasculature, with stenosis that “nearly totally occludes the lumen, along with severe pulmonary congestion and edema which may be related to acute or chronic heart failure.” Samples of the terminal aorta and iliac vessels of this animal revealed nearly complete stenosis of the lumen by gross examination (Fig. 3). All the other animals survived until the end of the experiment.

3.2. Collagen and elastin content

Of the 69 sealed samples, 31 yielded enough tissue to perform collagen and elastin quantification. The mean collagen amount was 52 μg/mg of tissue, and the mean elastin amount was 93 μg/mg of tissue.

3.3. Performance in atherosclerotic vasculature

Of the 69 seals (53 in atherosclerosis-induced animals and 16 in the control animal) evaluated, 64 were considered sealed, and five were considered not pathologically evaluable (seal not captured on the slide). There were two “regress” alarms necessitating a second attempt at sealing. Of the evaluable seals from atherosclerosis-induced animals, 38 were observed to have atherosclerotic lesions immediately adjacent to the seal, and 15 did not have atherosclerosis lesions adjacent to the seal. The 13 seals without atherosclerosis at the seal site were femoral artery specimens with no observable atherosclerosis or grade I atherosclerosis covering only a small portion of the artery. Two sites appeared to be adequately sealed despite the presence of a mineral deposit(s) within the seal (Fig. 2). The mean burst pressure was 716 ± 352 mm Hg in the control animal and 650 ± 335 mm Hg in the atherosclerotic animals.

3.4. Statistical modeling: predictors of burst pressure

Pearson correlations revealed moderate associations between burst pressure and atherosclerosis or percent luminal occlusion ($\rho = -0.28, P = 0.02$), burst pressure and collagen content ($\rho = 0.49, P = 0.005$), and burst pressure and size ($\rho = -0.50, P \leq 0.001$). Atherosclerosis and collagen content were also moderately correlated with vessel size. However, collagen content and percent luminal occlusion were not significantly associated ($\rho = -0.23, P = 0.21$).
site-specific deposition of atherosclerosis, with larger vessels tending to be more occluded. In our study, vessel size confounded the relationship between luminal occlusion and burst pressure, and after controlling for vessel diameter, percent occlusion or atherosclerosis was not significantly related to burst pressure.

3.4.2. Hypothesis 2: controlling for vessel diameter, increases in collagen content are associated with higher burst pressure. Collagen content was significantly related to burst pressure univariately; each 10-U increase in collagen content was associated with a 22% increase in burst pressure (95% CI = 7%–39%, P = 0.005, R² = 0.24). After controlling for vessel size, collagen content was still significantly associated with burst pressure, although the magnitude of this relationship was slightly attenuated. Controlling for vessel diameter, for every 10-U increase in collagen content, burst pressure increased by 15% (95% CI = 0.2%–32%, P = 0.047, R² = 0.36). Controlling for collagen content, vessel diameter was also significantly associated with burst pressure, with each 1-mm increase in vessel diameter being associated with a 12% decrease in burst pressure (95% CI = 1%–22%, P = 0.03). In addition, size and collagen were significantly related; for each 1-mm increase in vessel diameter, collagen content decreased by 3.4 U (95% CI = 0.4%–6.4%, P = 0.03, R² = 0.16). This may be because of inherent differences in collagen content between large iliac vessels proximal to the aorta and the smaller, more peripheral femoral vessels. In our study, size confounded the relationship between collagen content and burst pressure, and after controlling for size, the magnitude of the effect of collagen content was reduced, but still statistically significant. As hypothesized, increasing collagen content was associated with higher burst pressures.

3.4.3. Hypothesis 3: collagen will mediate the relationship between atherosclerosis and burst pressure. Because luminal occlusion was not found to be associated with burst pressure when controlling for size, mediation effects of collagen were not assessed. In addition, because luminal occlusion and collagen content were not significantly associated, collagen could not mediate the relationship between luminal occlusion and burst pressure. This lack of association may be because of our limited sample size.

3.4.4. Sensitivity analysis. Further analysis (Fig. 4) revealed a data point exhibiting high leverage; however, there was no experimental reason to remove this data point from the analysis. A sensitivity analysis excluding this data point showed no significant relationship between collagen content and burst pressure after controlling for vessel size (range, 2.1–7 mm). Excluding the outlier, controlling for size, for every 10-U increase in collagen, burst pressure increases by 8% (95% CI = −6.2%–24.5%, P = 0.27, adjusted R² = 0.26). However, size was still significantly associated with burst pressure. Controlling for collagen content, each 1-mm increase in vessel diameter was associated with a 13% decrease in burst pressure (95% CI = 3%–22%, P = 0.02).

4. Discussion

This study applied a novel approach to understand the influence of disease state on the safety and efficacy of a common bipolar vessel sealing instrument, while controlling for variables which have been shown to influence burst pressure such as...
as collagen content and size [13,24]. Currently, no quantitative data that is burst pressure, are published for the performance of any EBSD in human vasculature. Similarly, there is no data published on the performance of bipolar vascular sealers in an atherosclerosis animal model or human atherosclerotic vasculature. These class II devices may be marketed without such testing as they qualify for the 510(k) process, and bench data demonstrating safety and efficacy in excised, non-perfused, healthy animal vessels meet the requirement for market clearance.

In this experiment, the Yucatan pig model demonstrated many of the major features of severe atherosclerotic disease seen in humans including calcification, a fibrous cap, luminal thickening, and foam cells (Fig. 2). The animals did not however display "incompressible vasculature" as observed in elderly patients with extensive vascular disease. Extreme aneurysmal hardening of the iliac arteries and associated loss of elasticity and distensibility as observed in elderly patients with a history of smoking and coronary artery disease, was not possible to replicate in these young animals. As such, despite the fact that we technically (as defined by the Story scale) achieved high-level atherosclerosis with calcification in a large portion of the sites, it was still possible to easily approximate and compress the opposing walls of the arteries, with adequate collagen to provide a robust amalgam and seal. The point at which the vasculature becomes too brittle and calcified to achieve adequate compression for amalgam formation remains to be determined. Another limitation was the limited sample size. Because of a larger than expected standard deviation, a larger sample size containing collagen and additional control seals would have been beneficial.

Our results revealed that after controlling for vessel size, atherosclerosis, as measured by maximum luminal occlusion, had no influence on burst pressure. We found that vessel diameter confounded the relationship between luminal occlusion and burst pressure. This may be because of the site-selective susceptibility of lesion development in the large iliac vessels near the aortic terminus relative to the peripheral femoral vessels. Other authors have described similar site preference in animals and humans [29,30]. In addition, collagen did not mediate the relationship between atherosclerosis and burst pressure. In contrast to previous findings in rabbit models and in studies of human vasculature, collagen content was not significantly associated with atherosclerosis [31,32]. One potential reason that collagen content and luminal occlusion were not related in this study may be the effect of balloon catheter denuding on collagen levels. Other authors have demonstrated a 50% increase in collagen content as measured by hydroxyproline levels after balloon catheter denuding of the endothelium of carotid arteries in a healthy rat model after 60 d [33]. Similar results have also been observed in the iliac arteries after endothelial denuding in a healthy rabbit model [34]. The balloon catheterization of the porcine arteries and the resulting vascular injury may have caused similar increases in collagen content in our study across all the vessels irrespective of atherosclerotic state. This vascular remodeling may have canceled any effect of early atherosclerosis on vascular collagen content. Future investigators using this model should consider this potential confounding effect in their experiments.

We also found that increasing collagen content is associated with higher burst pressure. Controlling for vessel diameter, this relationship is slightly attenuated but still statistically significant. In this study, vessel size acted as a confounder in the relationship between collagen content and burst pressure, because larger vessels also tended to have lower collagen content. This may be because of inherent differences in collagen content between large iliac vessels proximal to the aorta and the smaller, more peripheral femoral vessels. It is established that collagen and elastin contents vary in canine's functionally discrete vascular or arterial beds [35,36]. We have also demonstrated that differing porcine arterial types exhibit markedly different collagen content and associated burst pressure measurements [13]. Other authors have shown that veins (which have lower collagen content) seal at a lower pressure than arteries [37]. Similarly, the functional distinction of iliac arteries proximal to the terminal aorta relative to the peripheral femoral arteries may be reflected in their differing collagen content.

Based on this experiment and previous work, it seems clear that although size contributes to burst pressure, it is not the sole determinant [13]. We have previously reviewed published testing methodologies and provided recommendations for a standard experimental methodology to reduce confounding and bias in assessing the efficacy of the devices [28]. From an animal model perspective, future investigators should note the tendency of site-specific plaque deposition in the iliac arteries and the increase in collagen content observed after balloon denuding and account for these factors in their experimental design. Further experiments with a larger sample size on human vessels with a range of atherosclerotic severity and age, sealed in vivo, are needed to better elucidate the confounding and mediational relationships between vessel disease state, morphology, and burst pressure. One solution for avoiding some of the confounding problems in the animal model is to use a single vessel type for testing. Human sample testing in a clinically relevant population may provide data for algorithm and instrument improvements tailored to patients with atherosclerotic disease. Similar experiments with ultrasonic devices are also warranted.

Acknowledgment

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References

Effect of collagen and elastin content on the burst pressure of human blood vessel seals formed with a bipolar tissue sealing system

Cassandra A. Latimer, MS, Meghan Nelson, BS, Camille M. Moore, MS, and Kimberly E. Martin, MSc

Department of Research and Development, Covidien Surgical Solutions, Boulder, Colorado
Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts
Department of Biostatistics and Informatics, University of Colorado Denver, Aurora, Colorado
Colorado Clinical and Translational Sciences Institute, University of Colorado Denver, Denver, Colorado

ABSTRACT

Background: Bipolar devices are routinely used to seal blood vessels instead of sutures and clamps. Recent work examining the impact of vascular proteins on bipolar seal performance found that collagen and elastin (CE) content within porcine arteries was a significant predictor of a vessel’s burst pressure (VBPr). This study examined seal performance across a range of human blood vessels to investigate whether a similar relationship existed. In addition, we compared VBPr and CE content between porcine and human blood vessels. Our primary hypothesis is that higher collagen-to-elastin ratio will predict higher VBPr in human vasculature.

Methods: In six cadavers, 185 blood vessels from nine anatomic locations were sealed using a bipolar electrosurgical system. A linear mixed model framework was used to evaluate the impact of vessel diameter and CE content on VBPr.

Results: The effect of CE ratio on VBPr is modified by vessel size, with CE ratio having larger influence on VBPr in smaller diameter vessels. Seal burst pressure of vessels 2–5 mm in diameter was significantly associated with their CE content. Comparison of average VBPr between species revealed porcine carotid and iliac arteries (440–570 mmHg) to be the best vessel types for predicting the seal strength of most human blood vessels (420–570 mmHg) examined.

Conclusions: CE content significantly modified the seal strength of small to medium sized blood vessels but had limited impact on vessels >5 mm.

1. Introduction

Bipolar electrosurgical devices are routinely used in open and laparoscopic surgical procedures to provide hemostasis to dissected tissue structures and blood vessels. Multiple evaluations have been performed to assess the seal strength, quantified through burst pressure, of different bipolar tissue sealing systems; however, these are significant deviations in reported measurements [1–5]. In general, porcine arteries and veins are used as a model for human blood vessels due to their
anatomic and physiological similarities. Some variation in the reported seal strength arises from the test method [6] or types of bipolar devices used, but when these factors are controlled, considerable differences remain. Further examination of published data found that the type of porcine blood vessel tested to evaluate seal strength differed by study and in some cases within a study.

Blood vessels from different anatomic locations have varying viscoelastic properties depending on their functional role [7,8]. The mechanical properties of blood vessels may be further influenced by genetics, age, lifestyle, and disease state [9–13]. Two primary components of vessel walls that have an important effect on the elasticity of blood vessels are elastin and collagen [14–16]. Prior work by this group examined the role of these structural proteins in porcine blood vessels and their influence on bipolar vessel seal strength [17]. A significant association between the ratio of collagen and elastin content (CE ratio) and seal strength as defined by vessel burst pressure (VBP) was found when controlling for vessel diameter; specifically vessels with larger CE ratios demonstrated greater seal strength. Conversely, no association was detected between vessel diameter and seal strength when controlling for CE ratio.

Given the limited published data measuring the seal strength of human blood vessels [18] and the lack of data comparing seal strength measurements between human and porcine blood vessels, it remains unclear whether porcine arteries are the best model for predicting the strength of human vessel seals. The objective of this investigation was to determine the relationship between human cadaver VBP and CE ratio and evaluate the suitability of porcine arteries as a model for human blood vessels. Our primary hypothesis was that in human cadaver blood vessels, higher CE ratio is associated with increased VBP and the strength of this relationship depends on vessel size. In keeping with Barlow’s formula (VBP = 2 × Strength × Wall Thickness/Diameter), we further hypothesized that the ratio of vessel wall thickness-to-diameter (WT/D ratio) may also contribute to VBP variability and impact the relationship between CE ratio and VBP. Finally, we qualitatively evaluated the suitability of various porcine arteries as a model for predicting the bipolar seal strength of human vessels by comparing the average VBP of human cadaver blood vessels observed in this study to those observed in prior studies of porcine arteries [17].

2. Methods

2.1. Human vessel collection and analysis of VBP

Our research was performed on six cadaveric subjects with donor consent obtained through Science Care (Phoenix, AZ). One female and five male subjects ranging in age (40–61 y), body mass index (BMI: 14–28), smoking status (yes, 2; no, 4), and who expired from various causes were evaluated. Cadavers were stored postmortem at 4°C until the time of dissection. Vessel harvesting from cadavers was performed within 3–11 d after death. Cadavers were dissected and nine types of vessels (carotid artery, deep femoral artery, femoral artery, iliac artery, inferior mesenteric artery, pulmonary artery, pulmonary vein, renal artery, and splenic artery) were evaluated. Bipolar tissue sealing systems are regularly used to seal the inferior mesenteric artery, splenic artery, and pulmonary arteries and veins in surgery. Although clinical sealing of carotid, femoral, renal, and iliac arteries would rarely occur, these vessels were included to compare with previously published data on the porcine vessel testing model and provide a range of vessel diameters and CE ratios to further elucidate the relationship between size, CE, and VBP.

Vessels segments were carefully dissected from surrounding connective and fatty tissue and their diameters were measured using white cotton string and a disposable ruler. Following diameter measurement, vessels were sealed in situ using a bipolar vessel-sealing system (Ligasure Atlas; Covidien, Boulder, CO). All seals were made with the standard two-bar setting on the ForceFrid generator system (Covidien) and one seal cycle per vessel was performed. After the vessel was sealed, the knife blade incorporated within the Ligasure Atlas device was activated creating two sealed segments. Before the dissection, one side of the seal was randomly selected for either burst test and collagen and elastin (CE) quantification or histologic examination. Sealed vessel segments including at least a 1-cm margin from the sealed tissue were removed from the cadaver for burst testing and histologic processing.

Sealed VBP was determined using previously described methods [2,17]. Briefly, a blunt tip cannula was inserted into the open vessel lumen and an iris was clamped around the vessel to contain infused water within the vessel lumen. Delonized water was injected into the vessel at a rate of 100 ml/min until the seal burst. Burst pressure was recorded using a pressure meter (Fluke; Everett, WA). The maximum VBP was recorded for each vessel tested.

2.2. Histologic analysis of vessel structure and CE content

Selected sealed vessel segments not subjected to burst testing were used for histologic analysis. After excision from the cadaver, the vessel samples were placed in 10% phosphate-buffered formalin for a minimum of 48 h before undergoing standard histologic processing. Samples were shipped to an independent histology laboratory (Premier Laboratory, LLC Boulder, CO) for sectioning, staining, and imaging. Histologic structure stains, hematoxylin and cosin and a modified Mason’s trichrome stain [19], were used to qualitatively examine the seal area, vessel structure, and CE content. Vessel wall thickness measurements were performed on modified Mason’s trichrome–stained samples.

2.3. Quantification of vessel CE content

After burst testing, vessel samples were placed into cryo tubes and stored in dry ice until they could be transferred to a −80°C freezer. Tissue sections were dissected from tissue adjacent to the seal. Care was taken during dissection to ensure all vessel layers were included in samples. Thawed tissue sections were weighed and transferred to microcentrifuge tubes for CE quantification. Total collagen was determined from tissue hydroxyproline content using the method described in
previous work [17]. Total elastin content, including soluble and insoluble elastin, was quantified using a commercially available kit (Fastin Elastin Kit; Biocolor Ltd., Belfast, UK). Following the kit instructions, duplicate measurements were made for each tissue sample and the final elastin concentration was expressed in μg elastin/mg tissue.

2.4. Data analysis

Differences in vessel diameter, CE ratio, and VBPr between vessel groups were examined using a Kruskal–Wallis test due to nonnormal distribution of the data. A P value <0.05 was used to determine statistical significance. Additionally, using 95% median confidence intervals (CIs), significant pairwise differences between vessel groups were evaluated.

To address our first hypothesis that CE ratio would predict VBPr, VBPr was modeled using a linear mixed model framework to account for the correlation due to repeated measurements taken on the same donors. VBPr was natural log transformed to satisfy the assumptions of linear mixed models. The primary explanatory variables included in the model were CE ratio and vessel diameter. An interaction between these variables was included to allow the effect of CE ratio on burst pressure to differ by vessel size. In addition, age, BMI, and smoking status were included as covariates in the model to control for donor-specific factors. A random intercept was used to account for between donor variability and for correlation between measurements made on the same donor. Sex was not included as a covariate in the model as only one donor was female, completely confounding the effect of sex and other sources of between subject variability, so that the impact of sex could not be estimated.

A similar linear mixed model framework was used to address our second hypothesis that WTD ratio could also influence burst pressure. Due to the smaller sample size (N = 75) available for this analysis and the large number of potential covariates, a backwards selection with a P to stay of 0.1 was used to develop a parsimonious model. In the model selection process, interactions were removed from the model before their associated main effects. Predictors considered in the model selection were CE ratio, vessel diameter, CE ratio by vessel diameter interaction, WTD ratio, and CE ratio by WTD ratio interaction, age, BMI, and smoking status.

All analyses were performed in R with the “nlme” package. Sensitivity analyses were performed to determine the influence of the data from the cadaver with a BMI of 14 on the results.

3. Results

3.1. Sample collection and exclusion criteria

A total of 223 vessel samples were obtained from the six cadaveric subjects in the nine previously listed vessel groups. To be included in the CE ratio and VBPr analysis, samples were required to have both a burst pressure measurement and CE quantification; 185 samples met these criteria. The excluded samples did not have burst test measurements due to technical difficulties during burst testing, such as equipment failure or the sample length being too short.

For the WTD ratio and VBPr investigation, 75 samples were available for analysis. Wall thickness measurements were performed on samples collected for histology. Because histology was only performed on selected samples, the sample size for this analysis was smaller than the CE ratio and VBPr analysis.

3.2. Histologic analysis of vessel structure and CE expression

A qualitative difference in the amount of collagen (blue) between different blood vessels was observed, with the splenic arteries displaying less collagen staining than the other vessels. Elastin content (black) did not appear to vary considerably between vessel types. Within the seal structure, vessel layers were compressed and most of the boundaries were not visible, but staining indicating CE remained, Figure 1.

3.3. Descriptive statistics

Mean and standard deviations for VBPr, CE ratio, collagen content, elastin content, and number of samples per vessel group are summarized in Table 1. A comparison of vessel diameter between vessel types using a Kruskal–Wallis test resulted in statistically significant differences (P < 0.001) between groups. Vessel groups that do not share the same letter have significantly different diameter sizes (95% median CIs), Figure 2. Performing a Kruskal–Wallis median test on the difference in median VBPr between vessel types also resulted in a significant difference (P = 0.001). Comparison tests conducted among vessel groups using the 95% median CIs showed a significant difference in median VBPr between all vessel groups, with the exception of the pulmonary artery group and the splenic artery group. Carrying out the same analysis on CE ratio resulted in a significant difference (P < 0.001). Deep femoral arteries had a significantly larger CE ratio than carotid, splenic, and pulmonary arteries; femoral arteries had significantly larger CE ratio than carotid and pulmonary arteries.

3.4. Hypothesis I: CE ratio and diameter as predictors of VBPr

Controlling for age, BMI, and smoking status, the effect of CE ratio on burst pressure was significantly modified by vessel size (P = 0.01). As vessel size increases, the effect of CE ratio on burst pressure decreases, Figure 3A. For example, for 2-mm diameter vessels, a 0.1 unit increase in CE ratio would result in a 5.9% increase in burst pressure (95% CI: 2.5% to 9.7% increase, P = 0.001); however, for a 6 mm vessel, the same increase in CE ratio would result in only a 0.8% increase in burst pressure (95% CI: 0.9% reduction to 2.7% increase, P = 0.4). The effect of CE ratio for a range of vessel diameters was modeled and is shown in Table 2. Although the impact of vessel diameter on burst pressure became stronger with increasing CE ratio, size was not a significant predictor of burst pressure for vessels with typical CE ratios between

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Fig. 1 – Blood vessel structure with a modified trichrome stain following sealing with a bipolar tissue sealing device. Blue stained fibers (collagen) and black stained fibers (elastin) vary by vessel type. Ca = carotid artery; DFe = deep femoral artery; Fe = femoral artery; II = iliac artery; IMA = inferior mesenteric artery; PuV = pulmonary vein; PuA = pulmonary artery; Re = renal artery; Sp = splenic artery. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

<table>
<thead>
<tr>
<th>Vessel group</th>
<th>VBPr (mmHg)</th>
<th>CE ratio (w/w)</th>
<th>Collagen (µg/mg)</th>
<th>Elastin (µg/mg)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep femoral artery</td>
<td>529 (249)</td>
<td>1.6 (0.6)</td>
<td>70.2 (19.5)</td>
<td>45.6 (13.0)</td>
<td>14</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>428 (206)</td>
<td>1.3 (0.5)</td>
<td>59.1 (16.5)</td>
<td>48.5 (12.5)</td>
<td>60</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>423 (131)</td>
<td>0.8 (0.3)</td>
<td>66.2 (13.6)</td>
<td>58.2 (11.3)</td>
<td>22</td>
</tr>
<tr>
<td>Iliac artery</td>
<td>459 (251)</td>
<td>1.1 (0.4)</td>
<td>64.5 (26.1)</td>
<td>56.3 (16.1)</td>
<td>12</td>
</tr>
<tr>
<td>IMA</td>
<td>564 (271)</td>
<td>1.1 (0.3)</td>
<td>54.9 (18.8)</td>
<td>54.0 (15.3)</td>
<td>7</td>
</tr>
<tr>
<td>Renal artery</td>
<td>480 (226)</td>
<td>1.0 (0.3)</td>
<td>59.0 (14.8)</td>
<td>60.1 (15.9)</td>
<td>26</td>
</tr>
<tr>
<td>Splenic artery</td>
<td>170 (69)</td>
<td>0.7 (0.3)</td>
<td>34.8 (16.0)</td>
<td>51.2 (8.6)</td>
<td>5</td>
</tr>
<tr>
<td>Pulmonary artery</td>
<td>320 (172)</td>
<td>0.8 (0.2)</td>
<td>48.9 (15.1)</td>
<td>65.6 (11.4)</td>
<td>20</td>
</tr>
<tr>
<td>Pulmonary vein</td>
<td>353 (183)</td>
<td>1.0 (0.3)</td>
<td>62.2 (20.1)</td>
<td>63.8 (11.4)</td>
<td>19</td>
</tr>
</tbody>
</table>

IMA = inferior mesenteric artery; w/w = weight/weight. Results are expressed as mean (SD) where applicable.
pressure is significantly modified by a vessel’s WTD ratio ($p = 0.046$). As the WTD ratio increases, the impact of CE ratio on burst pressure decreases, Figure 3B. For example, for a vessel with a relatively low WTD ratio of 0.1, burst pressure increases by 6.6% for each 0.1 unit increase in CE ratio (95% CI: 1.4% to 12.2% increase, $p = 0.01$). For vessels with higher WTD ratio of 0.2, this effect is attenuated, with burst pressure increasing by only 1.1% for each 0.1 unit increase in CE ratio (95% CI: 1.4% decrease to 3.6% increase, $p = 0.4$). In this study, the mean WTD ratio was 0.15, standard deviation = 0.05. For vessels in this range, burst pressure increases by 3.8% for each 0.1 unit increase in CE ratio (95% CI: 0.7% to 7.0% increase, $p = 0.02$), Table 3. Again, these results were robust to sensitivity analyses excluding data from the cadaver with low BMI.

4. Discussion

The objective of our investigation was to evaluate the relationship between the structural proteins CE and seal strength in human blood vessels. In terms of immediate utility, the aim was to use this information to determine which type(s) of porcine arteries were the most clinically relevant models for human blood vessels. This data may also drive improvements in device design or in generator algorithms. Our primary hypothesis was that in human cadaver vessels, higher CE ratio is associated with increased VBPr and that the strength of this association depends on vessel size. We found a complex
Table 2 – Estimated percent change in VBPr for a 0.1 increase in CE ratio for a range of vessel diameters.

<table>
<thead>
<tr>
<th>Vessel diameter (mm)</th>
<th>Percent change in VBPr for a 0.1 increase in CE ratio (%)</th>
<th>Lower 95% confidence limit (%)</th>
<th>Upper 95% confidence limit (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5.9</td>
<td>2.3</td>
<td>9.7</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>4.6</td>
<td>1.9</td>
<td>7.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>3.3</td>
<td>1.4</td>
<td>5.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>0.5</td>
<td>3.5</td>
<td>0.008</td>
</tr>
<tr>
<td>6</td>
<td>0.8</td>
<td>-0.9</td>
<td>2.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Furthermore, thick smooth muscle layers may affect the mechanics of the bursting process by altering the overall distensibility of the vessel. Including this parameter in future studies may assist with identifying a more accurate VBPr model and may lead to minor alterations in the device which could lead to improvements in seal quality in these types of vessels.

Bipolar seal strength was significantly different between human blood vessel types evaluated. The splenic artery, pulmonary artery, and pulmonary vein groups had lower average burst pressures than the other tested vessel types. Pulmonary blood vessels were anticipated to have lower burst pressures due to the range (4–30 mmHg) of normal physiological blood pressure exposure [20]. Limited data have been published on the physiological blood pressure of the splenic artery; one author reported the proximal back pressure of 15 splenic artery stumps to be 48.0 ± 9.8 mmHg [21]. Using this measurement, the average splenic artery burst pressure (170 ± 50 mmHg, Table 1) measured in cadaver tissue was at least three times the reported back pressure. The remaining human vessels tested, on average VBPr within 400–600 mmHg, Table 1. Comparing these average VBPrs with young porcine artery average VBPr, porcine iliac (440 mmHg) and carotid (670 mmHg) [17] arteries appeared to be the basest vessel models for predicting bipolar seal strength of most of the tested human blood vessels. Additionally, porcine femoral (270 mmHg) [17] and cadaver splenic arteries had similar average VBPr. Porcine renal arteries had a significantly higher average VBPr (1030 mmHg) [17] than all cadaver vessel groups tested.

Our study did have some limitations. A key difference between the porcine and cadaver investigations was the state of the tissue evaluated: living porcine tissue versus cadaver tissue. Clinical results in a living patient may not be identical.

Table 3 – Estimated percent change in VBPr for a 0.1 increase in CE ratio for a range of WTD.

<table>
<thead>
<tr>
<th>WTD</th>
<th>Percent change in VBPr for a 0.1 increase in CE ratio (%)</th>
<th>Lower 95% confidence limit (%)</th>
<th>Upper 95% confidence limit (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>6.6</td>
<td>1.4</td>
<td>12.2</td>
<td>0.01</td>
</tr>
<tr>
<td>0.12</td>
<td>5.5</td>
<td>1.2</td>
<td>10.0</td>
<td>0.01</td>
</tr>
<tr>
<td>0.15</td>
<td>3.5</td>
<td>0.7</td>
<td>7.9</td>
<td>0.01</td>
</tr>
<tr>
<td>0.18</td>
<td>2.1</td>
<td>-0.3</td>
<td>4.6</td>
<td>0.1</td>
</tr>
<tr>
<td>0.2</td>
<td>1.1</td>
<td>-1.4</td>
<td>3.6</td>
<td>0.4</td>
</tr>
</tbody>
</table>
to results in the cadaver model. We determined that using human vessels excised from a surgical procedure would not be feasible due to the limited number of excised vessels collected and the difficulty performing the burst pressure tests with these types of samples. Published data comparing the mechanical properties of cadaver, excised, and intact human blood vessels indicated that the viscoelastic, biochemical, and functional properties were similar between vessels among collection types [22–24]. As a result, we determined cadaver vessels would be an appropriate model for our study because a range of vessel sizes and types could be collected from a single subject and the mechanical properties would be comparable with living blood vessels.

Another issue with the cadaver model is the exposure of vessel seals to fluid pressure. Vessel seals created during in vivo porcine laboratories were immediately exposed to blood and seals could be judged on pass or fail criteria. Seals performed on cadaver tissue were only evaluated during burst pressure tests. If fluid pressure in situ does influence seal quality, we were not able to determine its impact on our study. To limit the effect of tissue degradation on blood vessel protein quantification and mechanical function, cadavers were stored at 4°C and we attempted to harvest and test blood vessels within a week after death. One donor was tested 11 d after death but protein content and VBP were not significantly different between this cadaver and the cadavers within our donor criteria. Therefore, vessels obtained from all subjects were combined into one data set.

The evaluations performed in both the porcine and cadaver tissue studies used the LigaSure vessel sealing system (Covidien). This system uses a proprietary closed-loop algorithm, which uses tissue impedance to determine energy delivery rate and seal completion. Consequently, the conclusions from these studies may have been influenced by the tissue sealing system and may not entirely be applicable to other bipolar technologies. However, because vessels from various anatomical beds have different mechanical properties, the circumferential force acting on any vessel seal would still be influenced by vessel structure itself. Thus, vessel structure likely has an effect on all energy sealed vessels but the extent may depend on the type of bipolar device used. Further elucidating the impact of tissue structure on seal performance could lead to the development of more reliable tissue sealing technologies that can tailor seals to tissue type. If it is not possible to improve bipolar sealing performance by altering mechanical and energy delivery algorithms alone, incorporating an exogenous substrate such as collagen could assist with sealing problematic tissue or vessels with thin walls such as the pulmonary artery and pulmonary vein. At the least, tissue structure information could be used by vessel sealing systems to determine the likelihood of seal success and could warn users who incomplete seals are more likely to occur.

New bipolar vessel sealing technologies are increasingly being approved for clinical use. Hemostatic efficacy is the most important function of bipolar tissue sealers. Standardizing the way these devices are evaluated [6] and having an appropriate model to determine seal performance is critical. Most of the young porcine artery types and cadaver blood vessels exhibited comparable seal performance; in particular, porcine carotid and iliac arteries and the majority of cadaver vessel groups evaluated had similar average VBP. In contrast, porcine renal arteries were the outlier group between studies, exhibiting much larger VBP than other porcine and cadaver vessel groups examined. In both comparison studies between technologies and in testing to gain regulatory clearance, care should be taken to ensure vessel types are clinically relevant and uniformly distributed between groups, so conclusions are not influenced by the vessel model.

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REFERENCES


APPENDIX E

COLLAGEN-ELASTIN RATIO PREDICTS BURST PRESSURE OF ARTERIAL SEALS CREATED USING A BIPOLAR VESSEL SEALING DEVICE IN A PORCINE MODEL

Sung Endore
DOI 10.1007/s00461-011-1606-4

Collagen–elastin ratio predicts burst pressure of arterial seals created using a bipolar vessel sealing device in a porcine model

David Sindram · Kimberly Martin · Jarrod P. Meadows · Ajita S. Prabhu · Jessica J. Heath · Iain H. McKillop · David A. Iannitti

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Abstract

Background Bipolar electrosurgical devices are used to generate rapid and efficient hemostasis in a wide range of surgical procedures. Of the factors that influence seal integrity, vessel (artery) diameter has been considered the most important variable. In this study we hypothesized that the relative ratio of the components that form the seal (collagen and elastin) determine the degree of vessel dis- tensibility and play an equally important role in defining seal strength.

Methods Porcine carotid, renal, iliac, and femoral arteries were sealed using a bipolar electrosurgical device in vivo. Following removal, arterial diameter was measured and vessels’ seals tested for arterial burst pressure (ABP).

Samples were then analyzed histologically and biochemically for collagen and elastin content.

Results Arteries with the highest collagen–elastin ratio (C/E) (renal) consistently demonstrated significantly higher burst pressures than those arteries with lower C/E ratios (iliac and femoral) independent of artery diameter.

Conclusion Using arteries of distinct anatomical origin and physiological function, we demonstrate that total collagen content, and more specifically C/E ratio, in porcine arteries is a more accurate predictor of ABP than vessel size alone.

Keywords Collagen · Elastin · Arterial burst pressure · Bipolar vessel sealing

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D. Sindram · J. P. Meadows · J. J. Heath · I. H. McKillop · D. A. Iannitti
Division of Hepatobiliary and Pancreatic Surgery, Department of General Surgery, Carolinas Medical Center, Charlotte, NC 28203, USA

D. Sindram · A. S. Prabhu · J. J. Heath · I. H. McKillop · D. A. Iannitti
Division of Gastrointestinal and Minimally Invasive Surgery, Department of General Surgery, Carolinas Medical Center, Charlotte, NC 28203, USA

K. Martin
Energy Based Devices, Covidien, Boulder, CO 80301, USA

D. Sindram (✉)
Department of General Surgery, Carolinas Medical Center, 1000 Byrhe Boulevard, Charlotte, NC 28203, USA
E-mail: David.Sindram@carolinashealthcare.org

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As such, distensibility/elasticity of the vessel wall may be an important factor in determining ultimate success of the specific vessel-sealing device employed.

With the absence of methodology to measure elasticity and distensibility of vessels directly in patients, vessel diameter (size of the vessel) has been used as the best indirect predictor of vessel seal success. Based on Laplace's law, vessel diameter is the largest contributor to the determination of the outward pressure on the vessel wall in a rigid system. Most vessel-sealing systems come with guidelines to limit the use on vessels over a certain size. However, failures of these devices are seen even when used on vessels well within the size limitation. In fact, very few vessels larger than 5–6 mm are routinely sealed using vessel-sealing devices. However, size may not be the best predictor of vessel-sealing success because it does not take the intrinsic vessel elasticity and distensibility into account.

Blood vessel elasticity/distensibility is intrinsically dependent on vessel wall structure, a factor that in turn is determined by multiple factors, including genetics, vessel thickness, age, and/or calcification diseases such as atherosclerosis [6–8]. An important aspect of vessel wall structure is the integration of elastin and collagen within the tissue [9–11]. Previous studies demonstrate that while the vessel wall comprises various components, collagen and elastin represent key factors in determining blood vessel elasticity [9, 10, 12]. Biochemically, collagen represents a more rigid structural protein whereas elastin is more flexible [12, 13]. The relative ratio of these two components thus plays a central role in determining blood vessel rigidity/elasticity. From a physiological standpoint, blood vessel elasticity is also dependent on anatomical location and vessel function [14, 15]. Since tension is directly proportional to the radius of the vessel wall (as formulated in Laplace’s law), the vessel wall construct is largely predictable by diameter and pressure (as described by Young’s modulus of flexibility). In addition to vessel size, location is similarly important. For example, the immediate surrounding tissue(s) plays an integral role in transducing pressures generated within the blood vessel wall. Similarly, certain vessels have evolved to demonstrate greater flexibility and resistance due to the inherent risks of physical damage dependent on location. For instance, carotid arteries are prone to injury due to the high degree of flexibility and rotation of the neck and repetitive stress on vessel walls, as well as exposure to external forces [10, 14]. It would therefore make sense that the collagen–elastin ratio may be different in carotids than that predicted on size and pressure alone. Conversely, visceral vessels of similar diameter may express different collagen–elastin ratios since they are not (naturally) exposed to the same degree of mechanical stress as in the carotid artery. Finally, biological function may also dictate the stiffness of a vessel to limit pressure variability. For example, the renal arterial system has a primary involvement in regulating the renin–angiotensin system and subsequent blood pressure modulation [16]. These observations are consistent with previous reports describing collagen–elastin content of major blood vessels [15].

A vessel seal is created using a bipolar vessel-sealing device by clamping tissue in between two electrodes and passing a current through the tissue. The impedance (electrical resistance) of the tissue results in agitation of ions and conversion of electrical energy into heat. The generator, to which the sealing devices are attached, continuously measures the tissue impedance (which changes during the sealing cycle) and changes the current through the tissue to optimize seal formation by following a specific algorithm. During the heating process, vessel wall proteins are irreversibly crosslinked, strengthening the seal. By avoiding excessive heating and charring, a more solid amalgam from denatured proteins is formed resulting in a stronger seal. The composition of the vessel wall with varying levels of collagen and elastin may, in turn, determine the strength of the seal itself.

Based on reports of vessel composition in canines [10, 15] and the variability associated with different tissue-sealing devices independent of vessel diameter alone [2, 4, 5], we hypothesized that collagen–elastin ratio in specific arterial blood vessels is directly related to burst pressure of arterial seals (ABPri) created using a bipolar vessel-sealing device (LigaSure™, Valleylab, Boulder, CO).

Methods

Animal model and surgical procedures

All studies and procedures were approved by the Institutional Animal Care and Use Committee of The Carolinas Medical Center and were performed in compliance with the Guide for the Care and Use of Laboratory Animals. Six female Yorkshire pigs (30–40 kg) were housed individually under standard conditions. Animals were allowed free access to food and water at all times, except for a period of overnight fasting prior to laparotomy. Animals were acclimated for 7–10 days prior to use. Pigs were given 5 mg/kg Baytril® intravenously 1 h prior to skin incision.

Pigs were placed in a supine position and monitored with pulse oximetry. General anesthesia was induced with 4.4 mg/kg Telazol®, 0.04 mg/kg atropine, and 1.5 mg/kg xylazine (given intramuscularly), and maintained with isoflurane inhalational anesthetic after endotracheal intubation. A full-length midline laparotomy incision was created, and the rectus abdominus muscles were dissected off of their origin at the pubis using Bovie electrocautery.
Once exposure was gained, the retroperitoneum was incised and the iliac vessels exposed proximally to the aortic bifurcation and distally to the inguinal ligament. In all arterial exposures, care was taken to leave the perivascular tissue intact to prevent arteries from retracting once divided. The midline incision was extended over the inguinal region and the inguinal ligament was divided, exposing the femoral vasculature. The renal arteries were next exposed bilaterally from their aortic origins until their distal branch points. Finally, bilateral neck incisions were made, and the carotid arteries exposed.

The LigaSure Atlas™ vessel-sealing system (Valleylab, Boulder, CO) was used to perform in vivo vessel sealing. All seals were made with a standard two-bar setting on the ForceTriad™ generator (Valleylab). The LigaSure Atlas vessel-sealing system was oriented with its jaws at a 90° angle to the vessel axis. Vessels were sealed and divided, using one complete seal cycle per vessel in a distal-to-proximal fashion to ensure blood flow through the vessel at the time the seals were created. The portions of the arteries proximal to the seals were controlled using silk suture ligature. A minimum of 1.5 cm of vessel was left between seal sites to allow adequate lengths for burst pressure testing.

Analysis of vessel diameter and arterial burst pressure (ABPr)

Following vessel sealing and harvest, arteries were processed by removal of fatty and connective tissue and vessel diameter was measured from the outside edge of the adventitia using digital calipers. Arterial burst pressure (ABPr) was then determined as previously reported [4, 5]. Briefly, a blunt-tipped metal angiocatheter (attached to pressure tubing) was inserted into the open lumen and secured to the angiocatheter with an iris clamp. The pressure tubing was in turn attached to a digital Fluke pressure monitor to record maximal intraluminal pressure (mmHg). A Cole-Parmer automated injection system was used to inject 0.9% (w/v) saline into the arterial lumen at a rate of 7.5 ml/min via a Y-connector in the pressure tubing. The maximal pressure was recorded as ABPr for a given vessel seal.

Histological analysis of blood vessel structure and collagen–elastin expression

Immediately following vessel diameter measurement, vessels were divided into two or more samples (depending on total vessel length). One sample was placed into a cryovial, snap-frozen in liquid nitrogen, and stored at −80°C prior to analysis for collagen and elastin levels. The second sample was placed into neutral-buffered formalin and stored at 4°C overnight prior to dehydration with serial ethanol cycles and embedding in paraffin. Paraffin-embedded tissue was then cut into 5-μm sections, mounted on slides, and deparaffinized. Sections were then stained using a modified trichrome stain [17].

Measurement of vessel collagen and elastin content

Immediately following vessel diameter measurement, arteries were cut into equal lengths (≈5 mm), placed into cryovials, snap-frozen in liquid nitrogen, and stored (−80°C) prior to analysis. Tissue was thawed to room temperature, wet weight (ww) determined, and the vessel divided equally for collagen and elastin analysis. Total collagen was determined from tissue hydroxyproline content based upon the method described by Edwards and O’Brien [18] and modified by Reddy and Ewemeka [19]. Briefly, samples (8–12 mg tissue (ww)) were hydrolyzed in 1 ml 6 N HCl (125°C, 15 ps) for 4 h. Samples were dried using a Savant SpeedVac (GMI Inc., Ramsey, MN) and reconstituted in 1 ml deionized water (dH₂O). Then a 25-μl aliquot was removed and the volume adjusted to 250 μl with dH₂O. Chloramine solution (100 μl) was added to an equal volume of the 1:10 hydrolyzed solution and allowed to incubate for 5 min at room temperature. One hundred microliters of 25.9% (v/v) perchloric acid was then added, and the tubes were vortexed and allowed to stand for an additional 5 min. p-Dimethylaminobenzaldehyde (DMBA, 100 μl) solution was added and each tube was vortexed and incubated for 20 min at 60°C before the addition of 600 μl ethoxyethanol. Samples were then vortexed and absorbance measured at 557 nm. Tissue hydroxyproline content was determined against a hydroxyproline standard curve as previously reported [19]. Tissue collagen was calculated by adjusting hydroxyproline content by the dilution factor, dividing by 0.13, and expressed as total collagen/mg tissue (ww). Duplicate tissue samples were used for each assay and absorbance for each tissue (hydrolyzate) was measured in triplicate.

Total elastin content was determined using a commercially available kit (Fastin Kit, Biocolor Ltd., Belfast, UK) as per the manufacturer’s instructions. Each vessel was analyzed in duplicate and data expressed as total elastin/mg tissue (ww).

Statistical analysis

Statistical analysis was performed using SPSS (Chicago, IL). A stepwise approach was enlisted to examine differences across artery type for variables of interest, i.e., collagen content, vessel diameter, vessel origin, and ratio of collagen to elastin (C/E). Correlations between predictor variables, with burst pressure as the dependent variable, were examined.
Results

Sample collection for analysis and exclusion criteria

Ninety individual arterial seals (femoral, iliac, renal, and carotid) were performed on six female Yorkshire pigs (30–40 kg) in vivo. All animals survived the duration of the study period. During the experimental protocol, five immediate seal failures occurred (5.6%) and were assigned a burst pressure of 0 mmHg. All burst pressures, including immediate seal failures, were included in statistical analyses. Four of the five seal failures occurred in an iliac or a femoral artery and one failure occurred in a carotid artery.

![Graph showing artery diameter](image)

**Fig. 1** Arterial diameter is not significantly different in porcine femoral, iliac, carotid, and renal arteries. Following vessel sealing, femoral (Fe), iliac (Il), carotid (Ca), and renal (Re) artery diameters were measured from the outside edge of the adventitia (of the nonsealed end of the vessel) using digital calipers. Results are expressed as mean ± SD.

Burst pressure measurements were obtained on 81 of 90 seals. The inability to obtain burst pressures on the remaining nine seals was attributed to technical difficulties such as an extracted arterial specimen that was too short to burst. All 90 arterial seal samples were evaluated for collagen and elastin content. Vessel diameter measurements were obtained for 86 samples (18 femoral arteries, 30 iliac arteries, 30 carotid arteries, and 12 renal arteries, Fig. 1). Although statistical analysis demonstrated differences between mean diameters of some of the artery groups (Supplementary Table 1), this parameter was not significantly correlated with the continuous variable ABPfr, either within a discrete arterial type or when considered continuously across all arteries. Measurements of the other four samples were not obtained due to equipment malfunction. Overall, there were 81 seals for which all parameters were collected.

Histological analysis of blood vessel structure and collagen–elastin expression

Sealed vessels were stained using a modified Masson’s trichrome stain [17]. This approach demonstrated normal arterial vessel architecture distal to the sealing site (Fig. 2). Extensive elastin (black) and collagen (blue) staining was detected in all blood vessels, with elastin staining more prominent toward the outside surface of the blood vessel (Fig. 2). At the seal site, blood vessel compression was extensive yet clear collagen–elastin staining remained visible (Fig. 2). Gross histological analysis demonstrated different elastin and collagen content depending on blood vessel type. While not quantified using a graded scale due

![Histological images](image)

**Fig. 2** Arterial collagen and elastin content is dependent on arterial type. Representative histological sections of femoral, iliac, carotid, and renal arteries stained using a modified trichrome stain following sealing using a bipolar vessel-sealing device. Using this technique, collagen fibers stain blue and elastin fibers stain black. Images were taken at low (×2, upper panels) and high (×10, lower panels) magnification. (Color figure online)
to subsequent empirical measurement of elastin–collagen content, we observed greater elastin (black stain using modified trichrome staining; Fig. 2) content in femoral arteries than in the other three types viewed. Conversely, collagen (blue stain using modified trichrome staining; Fig. 2) was relatively sparse in femoral and iliac arteries compared to that in renal and carotid arteries (Fig. 2).

Collagen–elastin ratios and blood vessel diameter as predictors of ABPr

Analysis of total collagen and elastin content in femoral, iliac, carotid, and renal arteries demonstrated an inverse correlation between total collagen and elastin expression in which vessels with the highest collagen content (renal, 120.7 ± 3.8 μg collagen/mg tissue ww) had the lowest elastin content (72.01 ± 7.79 μg/mg tissue ww) (Fig. 3). Conversely, those arteries with the lowest collagen (femoral, 72.2 ± 2.5 μg collagen/mg tissue ww) had the highest elastin (115.04 ± 10.61 μg/mg tissue ww) content (Fig. 3). These values were then used to generate mean C/E ratios for each vessel type, and these data, along with comparisons of relative artery size for each group, were compared against mean ABPr for each set of arteries (Fig. 3).

Having demonstrated the normality of the scale data, preliminary analysis using factorial ANOVA revealed overall significant differences (P ≤ 0.05) in every continuous variable by the categorical variable of artery type. Interestingly, the overall trend by arterial type in terms of mean ABPr and mean C/E appeared identical across different arteries analyzed and demonstrated the following trend: renal > carotid > iliac > femoral (Fig. 3). Post hoc analysis for differences in each of the variables between arterial groups was performed using Tamhane’s T2 test due to heterogeneity of variance in the overall data set. The results of this analysis are displayed by vessel type for each variable in Table 1. There were no serious outliers detected.

<table>
<thead>
<tr>
<th>Artery type</th>
<th>Equation to calculate ABPr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral</td>
<td>183 + (126 × C/E)</td>
</tr>
<tr>
<td>Iliac</td>
<td>301 + (126 × C/E)</td>
</tr>
<tr>
<td>Carotid</td>
<td>552 + (126 × C/E)</td>
</tr>
<tr>
<td>Renal</td>
<td>783 + (126 × C/E)</td>
</tr>
</tbody>
</table>

Table 1 Equations used to calculate arterial burst pressure (ABPr) of femoral, iliac, carotid, and renal arteries

Fig. 3 Collagen and elastin content predicts arterial burst pressure (ABPr). A Total collagen content was determined in femoral (Fe), iliac (Il), carotid (Ca), and renal (Re) arteries following vessel sealing and arterial burst pressure (ABPr) measurement. Values are expressed as mean ± SE; *P < 0.05 vs. Fe, †P < 0.05 versus Fe, Il, and Ca. B Total elastin content was determined in Fe, Il, Ca, and Re arteries following vessel sealing and ABPr measurement. Values are expressed as mean ± SE; †P < 0.05 vs. Fe, ‡P < 0.05 versus Fe, Il, and Ca. C Collagen–elastin ratio was calculated and plotted (C/E, left y axis) against mean ABPr (right y axis) for Fe, Il, Ca, and Re arteries following vessel sealing. Values are expressed as mean ± SE. Statistical analysis was performed and detailed in Supplementary Table 1.
An initial examination with a correlation matrix and exploratory linear models of the association of the independent variables with burst pressure reveals a Pearson correlation of $\rho = 0.59$ ($P < 0.001$, Fig. 4A) for total collagen and $\rho = 0.56$ ($P < 0.001$, Fig. 4C) for C/E, suggesting that these two variables alone are approximately equally linearly predictive of burst pressure in the absence of adjustment for any other fixed factors (Fig. 4). Vessel size was not significantly correlated with burst pressure, total collagen, or C/E in this initial analysis (Fig. 4). There was no significant correlation of vessel size with any of the variables of interest (Fig. 4 and Supplementary Table 1).

In considering the potential of C/E ratio, collagen, and artery type as predictors of the dependent variable of burst pressure, a stepwise linear regression was performed to determine how much of the variance in burst pressure can be attributed to these three variables with the following result: adjusted $R^2$ (79) = 0.53, $P < 0.01$. This analysis indicates that 53% of the variance in burst pressure can be predicted by this combination of variables and is a moderate to large effect (“grossly perceptible” and “visible to the naked eye” [20]). An individual examination of the contribution of each of the three variables to the burst pressure revealed that virtually all of the variance in the dependent variable of burst pressure was due to two variables: C/E [$R^2$ (79) = 0.32, $P < 0.01$] and artery type [$R^2$ (79) = 0.48, $P < 0.01$]. Adding total collagen into the linear model did not add to the predictive power.

Fig. 4 Collagen-elastin content and not vessel size is an accurate predictor of arterial burst pressure (ABP).

A Regression plot of total collagen content ($\mu$g of collagen per mg of wet weight tissue, $\mu$g/mg (ww)) versus arterial burst pressure (ABP, mmHg).

B Regression plot of total elastin content ($\mu$g/mg (ww)) versus arterial burst pressure (ABP, mmHg).

C Regression plot of C/E ratio versus arterial burst pressure (ABP, mmHg).

D Regression plot of vessel diameter (mm) versus arterial burst pressure (ABP, mmHg).

In an effort to better understand the individual contribution of the predictive variables to burst pressure, a standard linear regression was performed for the significant variable C/E, allowing for the differing variances in burst pressure by the categorical variable “artery type.” The regression equation for this analysis predicted that overall burst pressure can be calculated as follows: $783 + 126\text{C/E}$ ($\pm142$), with the following adjustments for artery type: subtract 231 for carotid ($\pm119$), subtract 600 ($\pm118$) for femoral, subtract 482 ($\pm112$) for iliac, and subtract nothing for renal as it was arbitrarily set as the reference level (Table 1).

Discussion

As early as 1966, physiologists described differences in the C/E ratio [15], and corresponding regional variation in elasticity, of arteries originating from functionally distinct vascular beds in a canine model [11, 14]. The results from this porcine study concur almost exactly with those studies in terms of regional variation in C/E. In addition, this study demonstrated a significant relationship between C/E expressed in comparably sized blood vessels and the corresponding arterial burst strength (ABP) following vessel sealing using a bipolar vessel-sealing device. Specifically, the collagen content relative to the elastin concentration in the wall of porcine arteries predicts, with high accuracy,
the burst pressure of vessels that are sealed and transected in a manner independent of vessel diameter.

Bipolar vessel-sealing devices have increasingly found their way into modern surgical practice [21]. Functionally, bipolar devices create vessel seals as a result of the heat generated when a current is passed through tissue, with the jaws of the device providing both the source of pressure (on the tissue) and the means to measure and regulate current flow. The combination of compression and heat causes proteins within the tissue to denature and subsequently re-associate, to form an amalgam [2, 21]. An effective seal is thus dependent on a sufficient amount of collagen (the more Hookean protein) from which to create the seal, and an optimal amount of elastin for flexibility in the distending vessel under pressure. The amount of collagen and elastin between the instrument jaws depends on the functional origin of the vessel. Iatrogenic factors such as the extent of vessel skeletonization and the amount/placement of tissue between the jaws will also affect the impedance and the energy delivered for a given seal.

The results of this study suggest that the overriding contributing factor is the optimal C/E ratio that correlates with optimal distensibility and higher burst pressures.

Previously, the major predictor of burst pressure and vessel-seal success was the diameter of the artery involved [22, 23]. In our experiments, it has become apparent that the major determinant of the strength of an arterial seal using the bipolar vessel-sealing device is the C/E content of the arterial wall, not vessel diameter. In our experiments we attempted to control for vessel diameter by choosing vessels of approximately equal size (femoral, iliac, carotid, and renal) (Fig. 1). Although the mean diameters of some of the arterial types did reach statistical significance, no significant correlation between size and burst pressure, or between size and any of the other parameters, was identified (Fig. 4D). This novel finding contradicts the long-held notion that vessel diameter is the most important predictor of burst pressure.

Other studies have demonstrated that the burst pressure of a vessel is a function of both the quality of the seal and the innate elasticity of the vessel [1, 4, 21–23]. Given the biochemical means by which vessel seals are formed, the ratio of the specific proteins (C/E) that undergo denaturation provides a logical explanation of why the C/E ratio is such a good predictor of seal quality, and thus burst pressure. Conversely vessel elasticity/distensibility is a function of the biophysical properties of the interrelated constituents of the artery, most notably the endothelium, smooth muscle, elastin, and collagen. Each of these constituents has a particular Young’s modulus value, and each contributes to the elasticity of the vessel to varying degrees [10].

The biomechanics of a sealed artery under pressure can be described by Laplace’s law \( P = \pi r^2 \), where \( r \) is the radius of the cylinder [artery], and \( P \) the pressure of the fluid within the artery. At physiologic pressure, elastin fibers are recruited and allow some deformation of the vessel, described by the Young’s modulus of elastin. However, at higher, supraphysiologic pressures, collagen fibers are recruited and, with a Young’s modulus several hundred times less than that of elastin, the tension in the vessel wall rises rapidly with little additional deformation until a critical pressure is reached and the vessel bursts [10]. Thus, considering Laplace’s law, one would predict that vessel diameter would have a large impact on the maximum burst pressure, and, in fact, logic dictates that there is a critical point at which diameter would contribute substantially to the predictive model. However, when one factors in collagen and elastin content, vessel functional origin, and the range of diameters examined in this study (2–5 mm), vessel diameter appears to be overcome by collagen and elastin content and vascular origin as a predictor of burst pressure. These findings are logical when one considers the physiologic functionality of renal and carotid vessels compared to the more peripheral vessels (iliac and femoral). A relatively low index of distensibility, correlating with a high C/E ratio, is necessary for vessels intimately involved in maintaining homeostasis. That is, excessive dilation of renal vessels would result in marked changes to the renin–angiotensin feedback loop [16], and similarly for the role of the carotid arteries-baroreceptors in regulating systemic blood pressure [11].

The difference in seal quality and burst pressure by vessel type is of further interest when considering energy-based ligation devices that use closed-loop control algorithms. In general, these devices detect the impedance of a given vessel and deliver energy (low current and high voltage), with the amount of energy being governed by a microprocessor algorithm. In this instance, the impedance is continuously monitored and the energy delivered is regulated until a critical value is reached and the device is automatically shut off. This design functions ostensibly to reduce sticking and charring at the site of contact to increase surgical efficiency. However, the data presented in this report suggest that altering these algorithms (in a vessel-specific manner) or adding collagen to the seals (to optimize the C/E ratio) may improve performance even further.

While data presented in this report demonstrate that the C/E ratio is more relevant than blood vessel diameter in determining seal integrity, other important factors specific to this study must also be considered. First, the data presented herein was obtained from studies performed in healthy, young animals. Loss of artery elasticity and distensibility due to aging may be important in altering the C/E ratio and thus the success rate of bipolar vessel-sealing
devices. Similarly, formation of the seal per se is dependent on protein denaturation/amalgamation formation across the blood vessel wall. In healthy animals, bringing the vessel walls within close proximity is (relatively) easy. However, this does not address the potential clinical setting, particularly in older patients, of atherosclerotic plaque formation. In this setting, bringing the vessel walls within close enough proximity to form the necessary protein amalgamation/seal is likely to be considerably more difficult. As such, future experiments in atherosclerotic animals are required to elucidate the effects of calcifications and atherosclerosis on the integrity of vessel seals and the relationship of collagen and elastin with vessel seal strength in this population. Next, our studies were performed using only the LigaSure bipolar device. Several similar devices are commercially available, each of which claims advantages over the others. As such, innovations and differences between the devices and the physics/biochemistry of how the seals are formed may similarly affect the role of C/E ratio in regulating seal strength. For example, the type of surface on the face of the sealing device is important in determining both seal strength and thermal spread [24]. On the one hand, using structured surfaces decreases the rate of seal failure compared to smooth surfaces, yet instrument sticking and thermal spread significantly increase when using structured surfaces [25]. Data from our study support the concept that structured surfaces may provide better seals due to the ability to bring vessel walls together more efficiently such that protein denaturation/amalgamation formation occurs. However, to define the C/E ratio as the most important factor in determining seal strength, identical studies to those presented herein should be performed using other devices to demonstrate that C/E ratio supersedes both vessel diameter and instrument type in determining seal strength.

Clinically, surgeons have little control over the size of the vessels that need to be sealed, or the C/E ratio of these vessels. Indeed, most surgeons intuitively will not seal large vessels, and most vessels that are sealed routinely are well within the recommended guidelines. However, the identification of collagen and C/E ratio as important determinants of burst pressure offers multiple opportunities to alter the sealing process and potentially improve vessel-sealing success. By altering or adjusting the generator algorithm based on the C/E ratio of the blood vessels that are being sealed, as predicted by location and perhaps age or disease state, a stronger amalgam and seal can perhaps be created. In addition, adding extraneous collagen to vessel seals may render the seals stronger as well. Further studies are required to quantify the efficacy of these interventions.

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References

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APPENDIX F

IN SEARCH OF THE AUTOLOGOUS CLIP: A CASE FOR EXPERIMENTAL STANDARDIZATION

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In Search of the Autologous Clip:
A Case for Experimental Standardization

Kimberly A. Krugman, MS, Kimberly E. Martin, BS, Ned Cosgriff, MD, and Douglas P. Slakey, MD, FACS

Abstract

Background: In an effort to enable faster and, at times, more challenging surgeries without compromising patient or physician safety, medical device manufacturers have created myriad solutions to vascular ligation through the development of novel tools. The speed of development, FDA approval, and dissemination of these devices into the hands of surgeons often outpaces the ability of investigators to critically evaluate comparative effectiveness of these devices.

Database: The Medline database was searched for energy-based vessel ligation devices. To remove any perception bias against non-Covidien instruments, critical review was applied only to the devices manufactured by our company.

Conclusions: We report on the variability present in published results and offer vital metrics for future studies. Standardized testing and reporting for measures of safety and efficacy of these surgical instruments await definition from a consensus group.

Introduction

Since the earliest recorded history, achieving and maintaining hemostasis has been important for successful surgery. The use of heat to staunch the flow of blood has been instrumental in furthering the ability of the practicing surgeon to take on more challenging cases. The application of heat in the surgical setting dates back to as early as 3000 BC, but conductive methods of heat transfer can result in extensive collateral tissue damage that overshadows benefits due to the uncontrolled nature of the tissue/energy interaction. A brief synopsis of the surgical energy evolution follows.

Monopolar electrosurgery

In the beginning of the 20th century, alternating electrical current found a number of applications in surgery due in large part to the work of French scientist d’Arsonval. His research demonstrated that alternating current could be passed through tissue without producing any physiologic effects outside of heating, and that if the frequency of alternating current was high enough, the unwanted side effect of muscle stimulation and contraction did not occur.

Collaboration between Dr. William Bovie and Dr. Harvey Cushing introduced high frequency monopolar electrosurgery to clinical use. The original Bovie generator delivered alternating current of very high frequency that created heat by ionic agitation (kinetic energy) at the tissue-electric interface. The heat affects proteins in vascular walls and plasma, creating a coagulum. Early monopolar devices did not provide for precise control of energy delivered to tissues, and unwanted collateral tissue damage, including carbonization, was a concern.

Bipolar electrosurgery: Introduction

In the 1940s, bipolar electrosurgical instruments were developed. Bipolar instruments allowed surgeons to direct the application of electrosurgical energy to specific tissue held between the electrodes of the instrument, usually in the form of forceps. Bipolar electrosurgery offered potential benefits over monopolar: (1) no return electrode needed to be attached to the patient, (2) effective at lower energy levels, minimizing collateral tissue damage, (3) coagulation in wet fields, and (4) compatible for patients with pacemakers, and implantable defibrillators.

1Department of Medical Affairs, Covidien Energy-Based Devices, Boulder, Colorado.
2Department of Surgery, Tulane University School of Medicine, New Orleans, Louisiana.
Bipolar electrosurgery: closed-loop control, first improvement

Continuous microprocessor feedback, along with the resultant modulation of thermoelectric output is at the heart of the so-called closed-loop control systems. Closed-loop control systems in electrosurgery sense changes in tissue impedance and adjust the output energy accordingly—much like cruise control on an automobile. Energy output increased in response to increased tissue independence (Ohm's law), and real-time power adjustments allowed lower output settings to be used, limiting collateral damage.

Bipolar electrosurgery: vascular ligation, present state

The introduction of a foreign body (suture, clips, etc.) was known to increase the patient’s potential for infection, and the possibility of using closed-loop control to create an autologous clip composed of the patient’s own proteins for vascular ligation was tremendously compelling. Kennedy et al. were the first to study the use of closed-loop bipolar electrosurgery for vascular ligation. Working with the variables of electrical current, bipolar jaw pressure, and energy delivery time, this group developed and subsequently optimized a bipolar energy system that was able to reliably seal vascular structures. They validated the system by measuring acute vascular burst pressure. The generator they developed sensed the characteristics of the tissue being sealed and provided a customized output algorithm for optimized energy delivery. Once the endpoint of the energy delivery algorithm was reached, the generator automatically ceased delivering energy and the user was alerted of this via an audio signal.

Today, surgical hemostasis is often achieved using energy-based device technology that relies upon complex, computer-controlled, impedance-based, automated feedback algorithms. It is essential that new technology be evaluated in a consistent and objective manner that allows the relative effectiveness of competing technologies to be elucidated. Unfortunately, the use of consistent experimental methods for evaluating the safety and efficacy of energy-based devices has been the exception rather than the rule. Considering that every surgeon uses electrosurgery during nearly every operative procedure, this lack of consistency in the evaluation and comparison of electrosurgical devices is something that should be improved upon.

We propose four critical factors that should be considered when evaluating energy-based vascular ligation devices: (1) seal/tissue fusion reliability/repeatability, (2) usability, (3) thermal profile, and (4) cost/economics. This article reviews the results of published literature in which authors have evaluated the effectiveness of energy-based devices, with the purpose of identifying the best methods of determining device safety and efficacy.

Device Evaluation: Need for Standardization

Unfortunately, at present it is impossible to accurately compare the new energy-based ligation devices on the market based on the published literature. Reported data reveal significant variations in experimental design, data stratification, analysis, and collection, making the results difficult to interpret. Interestingly, even for FDA device approval, no standard testing methodology exists.

Of the four critical factors listed above, we intend to examine in detail items 1 and 3 (seal efficacy and thermal profiles). To better understand the contributing factors leading to the reported variations, all device companion articles that (1) include Ligasure® instruments, (2) were performed in a bench model or in vitro, and (3) described a metric of acute seal efficacy were used to analyze experimental design, methods, models, and analysis. We searched the Medline database for studies that met the above criteria with the search terms of “Ligasure” OR “bipolar vessel sealer” OR “EBVS [electronic bipolar vessel sealing system]” OR “EBVS [electrothermal bipolar vessel sealing]” OR “vessel sealing” OR “vessel ligation” from 1996 to 2009. Table 1 lists the 12 articles that met our search criteria as well as the inclusion criteria.

Using this data set and exploring the range of reported burst pressure for all devices, the lowest reported mean burst strength is 128 mmHg and the highest reported burst is 1261 mmHg. This large discrepancy will be evaluated in the following sections, starting with an overview for sources of variation. A possible explanation for the inconsistency in reported data is the inherent heterogeneity of the methods employed for testing with respect to (1) definitions of failure and success criteria; (2) experimental design; and (3) variables considered in the data analysis (biologic/anatomic, user, device, etc.). A standard experimental methodology and a consistent nomenclature may allow for more judicious evaluation of the safety and efficacy of hemostatic devices.

Methodology

Robust experimental design is essential for achieving repeatable results and controlling sources of variation when possible requires a priori knowledge of the sources. Each column in Table 1 is intended as a mandatory variable that should be described in sufficient detail for the experiment to be replicated. Each column also represents an opportunity to introduce variation or even bias into the data. The instrument type, generator used, and settings selected can impact the outcomes; as many of these instruments employ different technology, comparisons should be made under the conditions of the manufacturer’s instructions for use. The animal model selected has predominately been porcine and the impact of introducing a new species type has not been studied. Performing sealing experiments in a bench setting may allow some environmental control yet negates the effects that perfusion imparts on the intended use and potential differences in data have not been studied to determine an optimal setting. There has been a remarkable lack of comment in the literature on the subject of whether the vessels sealed were completely or partially skeletonized. For reasons described below, the presence or absence of additional tissue on a vessel may introduce a substantial amount of variability across seals.

Iatrogenic factors may influence results across experiments; however, a more imperceptible source of variability recently described by Sindram et al. (accepted for publication) has been only cursory addressed in the literature. Variability arises from the anatomic and biomechanical properties innate to arteries arising from functionally distinct vascular beds, as well as the innate differences between arteries and veins. Failure to control for this biologic variability can result in biased conclusions. Briefly, the findings of Sindram et al. found a significant relationship between the collagen and...
<table>
<thead>
<tr>
<th>Reference</th>
<th>Instruments, generator, settings</th>
<th>Model</th>
<th>Setting</th>
<th>Vessel type</th>
<th>Sample size</th>
<th>Arteries</th>
<th>Vessel size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kennedy et al.2</td>
<td>Prototype</td>
<td>Fceine</td>
<td>Bench-abdominal</td>
<td>NS</td>
<td>Described</td>
<td>Both</td>
<td>Stratified by small (1-3 mm), medium (3.1-8 mm) &amp; large (5.1-7 mm) as well as arteries and veins</td>
</tr>
<tr>
<td>Goldstein et al.13</td>
<td>NS</td>
<td>Fceine</td>
<td>Bench-fresh excised</td>
<td>Urter</td>
<td>Described</td>
<td>NA</td>
<td>NR</td>
</tr>
<tr>
<td>Carbonell et al.12</td>
<td>Partially described</td>
<td>Fceine</td>
<td>Neck or abdomen</td>
<td>Partially described</td>
<td>A</td>
<td></td>
<td>Stratified by small (2-3 mm), medium (4-5 mm) &amp; large (6-7 mm)</td>
</tr>
<tr>
<td>Harold et al.4</td>
<td>Partially described</td>
<td>Fceine</td>
<td>Neck or abdomen</td>
<td>Described</td>
<td>A</td>
<td></td>
<td>Stratified by small (2-3 mm), medium (4-5 mm) &amp; large (6-7 mm)</td>
</tr>
<tr>
<td>Rajkovic et al.22</td>
<td>Reported</td>
<td>Fceine</td>
<td>Carotid</td>
<td>Described</td>
<td>A</td>
<td></td>
<td>Mean diameter of 5.0 mm for all</td>
</tr>
<tr>
<td>Lamberton et al.8</td>
<td>Reported</td>
<td>Bevine</td>
<td>Bench-fresh/thawed</td>
<td>NS</td>
<td>Described</td>
<td>A</td>
<td>5 mm</td>
</tr>
<tr>
<td>Newcomb et al.3</td>
<td>Reported</td>
<td>Fceine</td>
<td>Carotid, axillary, renal, iliac</td>
<td>Described</td>
<td>A</td>
<td></td>
<td>Stratified by small (2-3 mm), medium (4-5 mm) &amp; large (6-7 mm)</td>
</tr>
<tr>
<td>Landman et al.3</td>
<td>Reported</td>
<td>Fceine</td>
<td>In vivo acute</td>
<td>Renal</td>
<td>Described</td>
<td>Both</td>
<td>Stratified by small (2-3 mm), medium (4-5 mm) &amp; large (6-7 mm) as well as arteries and veins Mean diameter is also reported.</td>
</tr>
<tr>
<td>Richter et al.7</td>
<td>Partially described</td>
<td>Fceine</td>
<td>In vivo acute</td>
<td>Renal and Splenic</td>
<td>Described</td>
<td>Both</td>
<td>NS</td>
</tr>
<tr>
<td>Richter et al.7</td>
<td>Partially described</td>
<td>Fceine</td>
<td>In vivo acute</td>
<td>Renal, Spleen, Splenopancreatic, mesentric</td>
<td>Described</td>
<td>Both</td>
<td>NS</td>
</tr>
<tr>
<td>Hruby et al.12</td>
<td>Reported</td>
<td>Fceine</td>
<td>Femoral, iliac, renal</td>
<td>Described</td>
<td>Both</td>
<td></td>
<td>Stratified by small (2-3 mm), medium (4-5 mm) &amp; large (6-7 mm) as well as arteries and veins Mean diameter is also reported.</td>
</tr>
<tr>
<td>Parsons et al.12</td>
<td>Reported</td>
<td>Fceine</td>
<td>In vivo acute</td>
<td>Peripheral and visceral</td>
<td>Described</td>
<td>Both</td>
<td>Mean diameter and SD for each category were reported.</td>
</tr>
</tbody>
</table>

NR, not reported; NS, not specified; NA, not applicable; A, artery; ■ = missing information.
elastin ratio of functionally different vascular beds directly corresponding to the burst strength of those vascular beds. These results should be replicated in a larger sample; however, this study suggests that a discrete analysis of burst pressure by artery type is essential, and that this variable should be controlled, particularly in device comparison studies to avoid biased conclusions. From a methodology standpoint, the variables that should be controlled and reported include instrument used, hardware setup (generator model, power settings, etc.), animal model, environment/setting (in vitro, bench, etc.), stratification of vessel type (femoral, renal, iliac, carotid, etc.), sample size, distinction between arteries and veins, and explanation/stratification of vessel size.

**Burst pressure**

A number of authors have compared the hemostatic safety and efficacy of bipolar electrosurgical and ultrasonic vessel sealing devices using the maximum burst pressure of sealed vessels as a surrogate for the efficacy of the seal. Burst testing has been demonstrated by cannulating the open end of a sealed vessel, constricting back flow via suture or an iris clamp, delivering fluid into the lumen behind (upstream of) the seal, and incorporating a digital manometer in-line with the fluid delivery to provide a pressure reading (Fig. 1). The application of burst testing is not unique to vessel ligation research and dates back to the early 1990s in studies of the strength of vascular anastomoses.

Interestingly, the minimum criteria established for safety and efficacy was likely established through an engineering mindset where a 3× factor of safety seemed reasonable (and potentially arbitrary), suggesting a “safe range” of 360-400 mmHg (120 mmHg systolic pressure × 3).x The clinical relevance and significance of this metric has not been established. In some studies where multiple devices are compared, a definitive definition of an adequate seal is frequently omitted. There is often a failure to include basic descriptive statistics such as the standard deviation, rendering comparison of respective mean burst pressures for individual devices futile (Table 2). The end conclusion of device comparison studies is typically that the device with the highest mean burst pressure over multiple seals is the most reliable. However, this is not always the case and failure to include a margin of safety can lead to misleading conclusions.

**FIG. 1.** Burst testing apparatus: infusion pump, pressure meter, and cannula. The cannula is mounted on an aluminum fixture where the cannula can be fed into the open end of a sealed vessel and an iris clamp used to prevent backflow of saline during fluid delivery.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Flow rate</th>
<th>Flow mechanism</th>
<th>Success/failure criteria</th>
<th>Analysis</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
<th>Failure rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kennedy et al.²</td>
<td>Tried to ~100 mmHg/s for arteries and 10 mmHg/s for veins</td>
<td>NS</td>
<td>Failures reported but criteria not explicit</td>
<td>Typical descriptive statistics, data stratified by dia. ranges, probability of burst &lt;400 mmHg calculated</td>
<td>Reported</td>
<td>Reported</td>
<td>NR</td>
<td>Reported</td>
</tr>
<tr>
<td>Goldstein et al.¹⁰</td>
<td>Not a burst pressure study</td>
<td>Syringe-manual</td>
<td>NS</td>
<td>Minimal descriptive statistics, data stratified by dia. ranges, basic comparative statistics</td>
<td>Reported</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Carbonelli et al.¹⁰</td>
<td>Not controlled</td>
<td>Syringe-manual</td>
<td>NS</td>
<td>Minimal descriptive statistics, data stratified by dia. ranges, basic comparative statistics</td>
<td>Reported</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Harold et al.⁴</td>
<td>Not controlled</td>
<td>Syringe-manual</td>
<td>NS</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Reported</td>
</tr>
<tr>
<td>Rajabu et al.¹⁴</td>
<td>NA—specimens held at 2 predefined pressures</td>
<td>NS</td>
<td>Failures defined</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Reported</td>
</tr>
<tr>
<td>Lamberton et al.⁶</td>
<td>NA—specimens held at progressively increasing pressures</td>
<td>NS</td>
<td>NS</td>
<td>Typical descriptive statistics, lost resolution due to step increases, basic comparative statistics</td>
<td>Reported</td>
<td>Reported</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>Newcomb et al.⁵</td>
<td>7.5 cc/s</td>
<td>Automated infusion pump</td>
<td>Failures—burst &lt; 50 mmHg</td>
<td>Minimal descriptive statistics, data stratified by dia. ranges, basic comparative statistics</td>
<td>Reported</td>
<td>NR</td>
<td>NR</td>
<td>Reported</td>
</tr>
<tr>
<td>Landman et al.⁸</td>
<td>NR</td>
<td>Pressure syringe</td>
<td>NS⁸</td>
<td>Minimal descriptive statistics</td>
<td>Reported</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Richter et al.⁷</td>
<td>Not a burst pressure study</td>
<td>NS</td>
<td>NS⁸</td>
<td>Minimal descriptive statistics</td>
<td>Reported</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Richter et al.⁷</td>
<td></td>
<td>IV Pressure Bag</td>
<td>Artery burst &lt; 300 mmHg, Vein burst &lt; 50 mmHg</td>
<td>Minimal descriptive statistics, data stratified by dia. ranges, basic comparative statistics of vessel characteristics as well as burst pressure</td>
<td>Reported</td>
<td>NR</td>
<td>NR</td>
<td>Reported</td>
</tr>
<tr>
<td>Hruby et al.¹²</td>
<td>NS</td>
<td>IV Pressure Bag</td>
<td>Artery burst &lt; 300 mmHg, Vein burst &lt; 50 mmHg</td>
<td>Minimal descriptive statistics, data stratified by dia. ranges, basic comparative statistics of vessel characteristics as well as burst pressure</td>
<td>Reported</td>
<td>Reported</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Person et al.¹¹</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Basic descriptive statistics, data not broken out to control for arteries/veins, basic comparative statistics</td>
<td>Reported</td>
<td>Reported</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

*²Burst pressure success/failure not specified.
NS, not specified; NR, not reported; = missing information.
Thermal profile

The thermal characteristics of these energy-based devices contribute to the overall safety and usability of each device. Two distinct types of thermal information have been addressed: jaw temperature of an active device and thermal spread (or thermal propagation) of heat along a structure being sealed. These two factors provide important safety data to the user; maximum jaw temperature needs to be considered in laparoscopic settings or in endoscopic/minimally invasive type settings, and thermal spread needs to be considered when operating a device close to critical structures that could be harmed via thermal conduction or convection. Minimizing jaw temperature and thermal spread is typically not as critical as providing efficacious seals; however, a priori knowledge of the device’s thermal profile will enable the surgeon to confidently use the device in their environment.

The reporting of thermal metrics has been sparse though nearly every burst strength article has included some type of thermal information. For many of the same biomechanical reasons listed above that contribute to variability in burst strength, these factors also impact the amount of thermal propagation measured. The three governing methods of heat transfer are conduction (direct contact), convection (fluid-based transfer), and radiation (negligible in the presence of the first two). Conduction is the most studied and reported type and is referred to most commonly as thermal spread or lateral thermal spread. The means of evaluating this parameter have been by activating the device on a structure within the jaws and then making a gross hand measurement or using histology methods to determine a more precise measurement based on tissue/cellular damage. Convective heat transfer can play a role in creating adjacent site injury by the actions of steam production or activating in a wet (blood or saline) field.

There is no consensus on how to report thermal characteristics or what to report. Some authors have reported thermal spread as the damaged tissue length from the cut edge (wherever the instrument was used to transect the structure) into healthy tissue, 10,12,15,16 whereas others have reported the length of tissue damage starting from the seal’s edge into healthy tissue. 10,11 In one case, the lead pathologist chose one method for thermal spread assessment in an early article 6 and then modified the method of measurement in their most recent article without adding a rationale for the modification. 12 Another group chose to use thermistors at a prescribed distance from the outside of the jaw to measure peak temperature but did not follow this with any histological assessment. 9 The measurement method becomes important when the type of technology used is considered. Devices such as LigaSure, BIClamp, and EnSeal 9 confine tissue between two jaws during the sealing process, whereas ultrasonic devices have an active blade that is open to the environment during the division process. Arguably, any tissue confined within the jaws of a vessel sealing device is tissue that the user expects to be sealed and thermal spread should be considered as damage that extends beyond the confines of the jaw (or sealed area).

Table 3 identifies the depth and breadth of thermal spread reporting from the Table 1 sources. Roughly 1/3 of all relevant information regarding thermal spread reporting is missing from these publications. The methods applied and stratification of the data demonstrate a lack of consistency as well as study design considerations. Some of the studies have shown a linear relationship between increasing vessel size and increasing thermal spread, 3,10 and other investigators have reported distinct differences between thermal spread on arteries and veins. 7,12 Using the LigaSure V as a test case, we found that the reported thermal spread ranged from as low as 0.4 mm 11 to as high as 6.3 mm 10 depending on the author’s methods as well as the model conditions.

The other type of thermal information useful for communicating safety is jaw temperature. Knowledge of peak temperature achieved during activation as well as the latent heat capacity of the jaws after activation enables the user to apply an energy-based ligation device in a safe manner for their environment as well as handle the instrument safely after application. While the notion of surface temperature mapping is not new, the application of this technology to energy ligation devices is still in its infancy. In 2003, Campbell et al. performed the first study to use intra-red camera monitoring for evaluating device thermal profiles. 12 Since this inaugural publication we found only three other published works covering the topic.18,20

The importance of understanding the overall jaw temperature is intimately tied to the usability of the device. The surgeon user should know the maximum temperature typically achieved by the jaws as well as the length of time required for the jaws to safely cool. These two metrics are indicators to the user for safe activation and safe movement postactivation. In the pursuit of recording and reporting the outcomes of thermal imaging, standardization continues to be requisite. Factors that can introduce variability to the data include tissue type used, ex vivo/in vivo setting, number of activations, open/laparoscopic environment, instrument (between manufacturers and within one manufacturer), and amount of tissue within the jaws. We encourage authors to carefully consider their sources of variation and control as many factors as possible in future investigations.

Conclusions

In summary, it is important that surgeons be critical in reviewing data and evaluating electrosurgical devices. The authors chose to use the LigaSure V as a test case for exploring the wide variability present in reporting benchmark performance of energy-based devices; this LigaSure device has the most published data of any LigaSure device. The lack of consistent testing methods and reporting of results makes the comparison of devices difficult. In addition, because there are not defined standards the true safety, efficacy and cost-effectiveness of these devices are equivocal. This review identifies the need for development of a common lexicon and standardized testing and performance evaluation of energy-based devices. The authors believe that a consensus group formed from practicing surgeons and surgical researchers would be the appropriate starting point for defining the appropriate clinical metrics of these important energy-based device parameters.

Disclosure Statement

K. Krugman, K. Martin, and Dr. Cosgriff are employed in the Medical/Clinical Affairs Department and receive salaries from Covidien. Energy-Based Devices, Dr. Staley has received honorarium from Energy-Based Devices for prior
### Table 3. Variable Reporting and Analysis Techniques Regarding Thermal Profile Data from Table 1 Publications

<table>
<thead>
<tr>
<th>Reference</th>
<th>Instrument, generator, settings</th>
<th>Model</th>
<th>Setting</th>
<th>Tissue type</th>
<th>Artery/vein</th>
<th>Vessel size</th>
<th>Mean thermal spread</th>
<th>SD thermal spread</th>
<th>Range thermal spread</th>
<th>Method reported</th>
<th>Histology used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kennedy et al.</td>
<td>Prototype</td>
<td>Porcine</td>
<td>Bench-abdomen</td>
<td>NS</td>
<td>NA</td>
<td>NA</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Goldstein et al.</td>
<td>Partially described</td>
<td>Porcine</td>
<td>Bench-fresh excised</td>
<td>Upper</td>
<td>NA</td>
<td>NR</td>
<td>Reported</td>
<td>NR</td>
<td>Reported</td>
<td>Reported</td>
<td>Reported</td>
</tr>
<tr>
<td>Carbonell et al.</td>
<td>Partially described</td>
<td>Porcine</td>
<td>Bench-fresh excised</td>
<td>Neck or abdomen</td>
<td>Both</td>
<td>Reported</td>
<td>Reported</td>
<td>NR</td>
<td>NR</td>
<td>Reported</td>
<td>Reported</td>
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<tr>
<td>Harold et al.</td>
<td>Partially described</td>
<td>Porcine</td>
<td>Bench-fresh excised</td>
<td>Neck or abdomen</td>
<td>Both</td>
<td>Reported</td>
<td>Reported</td>
<td>NR</td>
<td>NR</td>
<td>Reported</td>
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<tr>
<td>Rajaboli et al.</td>
<td>Partially described</td>
<td>Porcine</td>
<td>Bench-fresh excised</td>
<td>Carotid</td>
<td>A</td>
<td>Reported</td>
<td>No thermal spread measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamberton et al.</td>
<td>Partially described</td>
<td>Bovine</td>
<td>Bench-fresh/thawed</td>
<td>NS</td>
<td>A</td>
<td>Reported</td>
<td>Performed via thermistor at 2 mm</td>
<td>Reported</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newsom et al.</td>
<td>Partially described</td>
<td>Porcine</td>
<td>Bench-fresh excised</td>
<td>Carotid, axillary, renal, iliac</td>
<td>A</td>
<td>Reported</td>
<td>No thermal spread measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Landrum et al.</td>
<td>Partially described</td>
<td>Porcine</td>
<td>In vivo acute</td>
<td>Renal</td>
<td>Both</td>
<td>Reported</td>
<td>Generalized</td>
<td>NR</td>
<td>NR</td>
<td>Reported</td>
<td>Reported</td>
</tr>
<tr>
<td>Richter et al.</td>
<td>Partially described</td>
<td>Porcine</td>
<td>In vivo acute</td>
<td>Renal and spleen</td>
<td>Both</td>
<td>Reported</td>
<td>Generalized</td>
<td>NR</td>
<td>NR</td>
<td>Reported</td>
<td>Reported</td>
</tr>
<tr>
<td>Richter et al.</td>
<td>Partially described</td>
<td>Porcine</td>
<td>In vivo acute</td>
<td>Renal, splenic, subcapsular, mesenteric</td>
<td>Both</td>
<td>Reported</td>
<td>Generalized</td>
<td>NR</td>
<td>NR</td>
<td>Reported</td>
<td>NA</td>
</tr>
<tr>
<td>Hruby et al.</td>
<td>Partially described</td>
<td>Porcine</td>
<td>In vivo acute</td>
<td>Femoral, iliac, renal</td>
<td>Both</td>
<td>Reported</td>
<td>Reported</td>
<td>NR</td>
<td>NR</td>
<td>Reported</td>
<td>NS</td>
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<tr>
<td>Posus et al.</td>
<td>Partially described</td>
<td>Porcine</td>
<td>In vivo acute</td>
<td>Peripheral and visceral</td>
<td>Both</td>
<td>Reported</td>
<td>Reported</td>
<td>NR</td>
<td>NR</td>
<td>Reported</td>
<td>Reported</td>
</tr>
</tbody>
</table>

NR, not reported; NS, not specified; – = missing information.
participation on Surgeon Advisory Committees (Dr. Slakey was not compensated for his participation on this article).

References

Address correspondence to:
Kimberly A. Krugman, MD
Department of Medical Affairs
Covidien Energy-Based Devices
5020 Longbow Drive
Mail Stop A-15
Boulder, CO 80301

E-mail: kimberly.krugman@covidien.com