

PERCUTANEOUSLY CONTROLLED HYDRAULIC OCCLUDERS AND THEIR
APPLICATION IN GRADUAL VENOUS OCCLUSION

By

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Abstract of Dissertation Presented to the Graduate School
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Surgical occlusion is widely accepted as the treatment of choice for congenital portosystemic shunts in dogs. Unfortunately, acute occlusion of portosystemic shunts is frequently associated with life threatening portal hypertension. Despite years of research in this area, veterinary surgeons continue to search for a safe and effective method to produce gradual occlusion of portosystemic shunts in dogs.

The overall goal of this thesis project was to evaluate a novel technique for gradual venous occlusion using a percutaneously controlled silicone hydraulic occluder. In order to identify the qualities desired in an ideal surgical device for treatment of congenital portosystemic shunts, a comprehensive literature review pertaining to congenital portosystemic shunts in veterinary patients was performed.

Following the literature review, a prospective evaluation of the silicone hydraulic occluder was performed using a rat model. The vena cava of each rat was instrumented with a silicone hydraulic occluder, as well as a perivascular ultrasonic flow probe.

Gradual decrease in blood flow was documented during an 8-week period, although limitations were identified with the experimental model due to the size disparity between the large implants and the small, compressible rat vena cava. A second problem identified was a marked difference between the predicted filling volume of the occluder and the actual filling volume required to cause cessation of blood flow. It was hypothesized that several factors contributed to this phenomenon, including diffusion of filling solutions, size differences in the HOs secondary to manufacturing inconsistencies, and plastic deformation of the materials over time.

Due to questions regarding the reliability of the vascular occluders in chronic applications, a series of in vitro experiments were performed to evaluate the mechanical properties of hydraulic occluders immersed in simulated body fluid. Data from these studies confirmed that air diffused rapidly from the occluders, whereas both saline and sodium hyaluronate functioned as appropriate filling solutions. Size differences among the occluders secondary to manufacturing were statistically significant, and could potentially affect filling volumes and fine adjustment of the level of occlusion in chronic applications. Changes in internal pressure were also noted to occur over time, suggesting that mechanical deformation of the silicone occluders may also contribute to changes in filling volume required. Mechanical deformation appeared to be more significant in the smaller occluders studied.

CHAPTER 1
METHODS OF GRADUAL VASCULAR OCCLUSION AND THEIR
APPLICATIONS IN TREATMENT OF CONGENITAL PORTOSYSTEMIC SHUNTS:
A REVIEW.

Introduction

Gradual vascular occlusion was originally attempted in experimental models of human coronary artery disease and portal hypertension.^{1,2,3} While these experimental studies gave rise to a small number of clinical applications for surgical treatment of vascular anomalies in human patients,^{4,5} veterinary surgeons have performed numerous clinical and experimental studies evaluating methods of gradual vascular occlusion for congenital portosystemic shunts (CPS). The purpose of this manuscript is to review the reported clinical and experimental techniques of gradual vascular occlusion and to discuss their potential for application in the treatment of CPS.

Surgery is generally advocated for the definitive treatment of CPS in dogs and cats.⁶⁻¹⁵ Animals that survive the perioperative period have a good long-term prognosis, while animals treated with medical therapy undergo progressive hepatic failure.^{6,13} Although surgical attenuation of CPS has been performed in thousands of dogs over the past 3 decades, refinements in surgical techniques continue to evolve.

For many years, CPS were treated via ligation of the aberrant vessel with suture material. Unfortunately, acute complete attenuation of the shunt at the time of surgery often caused intolerable portal hypertension, and as a result, only 17% (4 of 24) to 55% (8 of 15) of single extrahepatic CPS, and 13% (15 of 114) of intrahepatic CPS could be completely ligated during a single surgical procedure.¹⁶⁻²⁴ It was felt that the poorly

developed hepatoportal vascular system was unable to cope with the sudden increase in blood flow. Guidelines, based on measurement of portal pressure^{16,25,26} and subjective visual criteria²³ during complete occlusion of the shunt were established to determine the amount of attenuation that could be performed during a single surgical procedure. Partial ligation was recommended when complete ligation could not be safely achieved during a single surgery.

Despite causing an early clinical improvement, it became evident that partial ligation of CPS was associated with a less favorable long-term prognosis than complete ligation.^{8,14,16,27} Adverse sequelae manifested by the central nervous system, gastrointestinal tract, urinary tract, and cardiovascular dysfunction were frequently associated with partial occlusion of CPS.⁸ Complete attenuation, as compared to partial attenuation, was superior in obtaining resolution of clinical signs and improving long-term survival.^{6,13,14,23,25,28}

Accordingly, some authors advocated performing a second surgery to attain complete occlusion of CPS when portal pressures precluded acute ligation during the initial procedure.⁶ It was suggested that if attenuation was performed over a period of 4 to 6 weeks, the liver would adapt to the redirection of normal portal blood flow.^{6,7,29} Multiple surgeries, however, introduced increased risk to the animal, greater morbidity, and increased costs.

In an attempt to avoid multiple anesthetic and surgical, most recent efforts have focused on identification of a surgical implant that could be used to produce gradual, and complete occlusion of CPS after a single surgical procedure. The ideal implant would produce reliable progressive venous occlusion, would be biocompatible, inexpensive,

simple to apply, associated with minimal patient morbidity, and would be reversible if postoperative portal hypertension were to occur. Methods of gradual vascular occlusion that have been employed in the clinical treatment of CPS include: partial ligation with silk suture material,^{6,8} ameroid constrictors (AC),⁹ cellophane banding,^{10,29} and intravascular deployment of thrombogenic coils (TC).¹² The silicone hydraulic occluder (HO) has also been used to produce gradual venous occlusion in experimental models, but its use in the treatment of CPS has not yet been reported. The purpose of this chapter is to provide a comprehensive review of the available experimental and clinical studies related to each method of gradual venous occlusion and to discuss potential advantages and disadvantages of each technique in the treatment of CPS in dogs and cats.

Silk Suture

Silk has long been utilized as a suture material for vascular ligation due to its excellent handling properties. This natural, multifilament suture is classified as a non-absorbable material due to its prolonged retention in tissues.³⁰ Silk suture material is capable of inducing an inflammatory response within a vessel wall.⁷ Acute inflammation characterized by chemotaxis and degranulation of neutrophils is followed by a more chronic response consisting of fibroblast infiltration, proliferation, and eventual scar formation.^{7,31-33} It has been suggested that the inflammatory response initiated after partial vascular occlusion with a silk ligature may eventually lead to complete vascular occlusion.⁷ In fact, various investigators have reported successful complete CPS occlusion, as determined by portal scintigraphy, after partial attenuation with silk.^{7,8,22,34} An experimental model using the femoral vein in dogs failed to corroborate these clinical observations.³⁵ Of the four methods of gradual venous

occlusion examined in that study, partial occlusion with silk was the sole technique that failed to produce appreciable venous attenuation over a 6-week period.³⁵

These discrepancies associated with the use of silk for progressive vascular occlusion may be related to several different factors affecting the degree of perivascular inflammation. The primary factor implicated in the variable rate of occlusion after partial ligation with silk suture is the degree of inflammation produced by perivascular dissection. Delayed vascular occlusion may be the result of excessive manipulation of the vessel during surgery, and the resulting postoperative vasospasm or swelling of the vessel wall.⁷ Surgically induced vasculitis may induce a transient narrowing of the vessel and decrease in blood flow during the immediate perioperative period.^{7,27,35} Some authors have postulated that variations in the degree of fibrosis associated with the use of silk ligatures are directly related to suture diameter.³⁶ Hunt and Hughes, however, found no significant difference in the proportion of animals experiencing complete occlusion after partial attenuation with different gauges of silk suture.²⁷ Another potential explanation for improved clinical status following partial occlusion with silk sutures is that occlusion is not actually produced by progressive attenuation of the vessel lumen, but rather by alterations in blood flow that predispose the shunt vessel to thrombosis. For example, partial re-direction of blood flow through the portal system may encourage hepatic regeneration and a reduction in portal vascular resistance to the point that shunting of blood through the attenuated vessel is no longer favored.³⁵ The resulting decrease in blood flow through the shunt may produce closure or thrombosis of the vessel secondary to stasis.

Advantages of partial CPS ligation with silk suture include widespread availability, and low cost of the materials. Silk is adaptable to both extravascular, transportal, or transcaval approaches to portosystemic shunt ligation,²¹ and is thus applicable for both extrahepatic and intrahepatic CPS. Disadvantages include the requirement for measurement of intraoperative portal pressures during CPS ligation and the potential need for a second surgical procedure to achieve complete ligation in many dogs with CPS. Although partial occlusion of CPS with silk suture appears to provide favorable short-term clinical results in select cases,⁸ the ability of silk to produce complete attenuation in a predictable manner has yet to be demonstrated.³⁵ The use of silk suture material in the treatment of extrahepatic CPS in dogs has, for the most part, been replaced by the application of ameroid constrictors or cellophane bands that can be placed during a single surgical procedure without measurement of portal pressures. Intrahepatic PSS, however, are still treated using partial ligation with silk when they are not amenable to placement of larger surgical implants.

Ameroid Constrictors

Ameroid, first described by Berman et al in the mid 1950's, is a hygroscopic substance consisting of compressed casein that is hardened via a formalin curing process.¹ The material expands after exposure to water. Ameroid constrictors were originally designed with the intent of producing physical compression of blood vessels, resulting in gradual vascular occlusion. The first ameroid constrictors consisted of large rings with eccentrically placed lumens. The eccentricity allowed one side of the ring to expand faster than the other, effectively filling the lumen and occluding the circumscribed vessel.² A second device utilizing ameroid was later described for slow progressive vascular occlusion, specifically as a method of banding the pulmonary artery

of infants.³⁷ This implant relied on an ameroid cylinder that acted as a piston as it expanded, pushing an inert plastic anvil against an adjustable polytetrafluoroethylene band and causing constriction of entrapped tissues.³⁷ The AC being used today for treatment of CPS contain an inner ring of ameroid within an outer stainless steel sheath (Research Instruments NW, Inc. Sweet Home, OR) A small cylindrical piece of ameroid functions as a key, which closes the ring once it has been placed around the vessel.

(Figure 1)

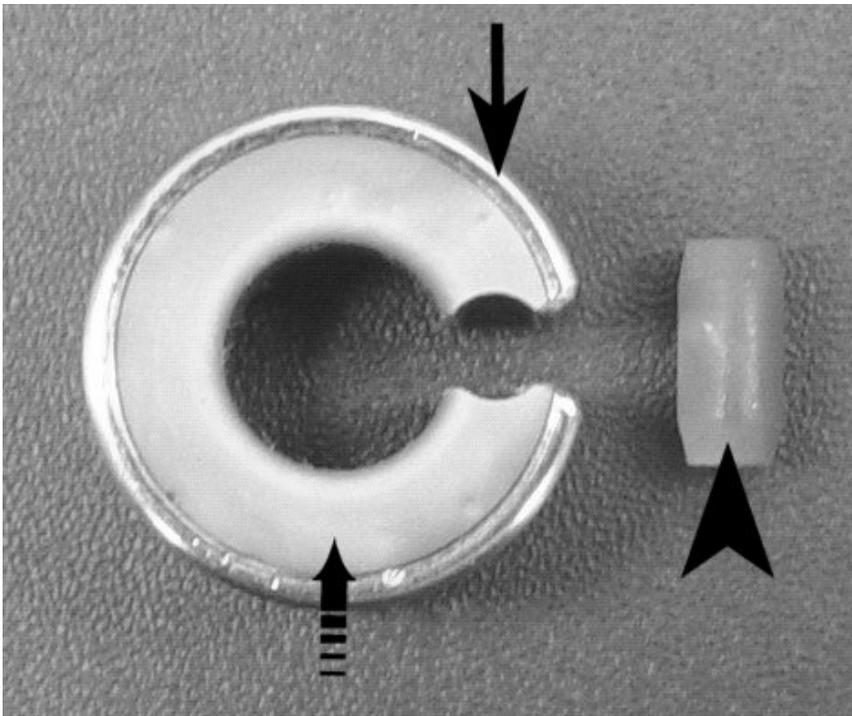


Figure 1. Photograph of a 5mm ameroid constrictor and key. An outer steel sheath (arrow) contains an inner ring of casein (dashed arrow) which forms a complete ring when the cylindrical key (arrow head) is inserted.

As the ameroid absorbs fluid, expansion is directed centrally by the stainless steel ring, resulting in a decrease in internal luminal area. Although size, shape, and stiffness of material encasing the ameroid, as well as the type and temperature of the surrounding fluid alter the rate of closure, a relatively consistent expansion pattern has been

demonstrated, occurring most rapidly in the first 3-14 days following implantation, and slowing thereafter.³⁷⁻³⁹

Ameroid constrictors have been used extensively in a variety of research applications for over 50 years, including experimental models of coronary stenosis,^{1,2,40} myocardial ischemia,⁴¹ limb ischemia,⁴² development of coronary collateral circulation,⁴³ esophageal varices,⁴⁴ and portal hypertension.⁴⁵ In 1996, Vogt et al reported the use of AC for treatment of single extrahepatic CPS in dogs.⁹ In Vogt's original study, complete attenuation of the CPS was documented in 10 of 14 animals using technetium scintigraphy. Complete occlusion was identified at times ranging from 30 to 210 days. There was, however, a 14% mortality rate due to portal hypertension, and 17% of animals developed multiple extrahepatic (MEH) portosystemic shunts, presumably due to portal hypertension that occurred during occlusion of the CPS. A subsequent study reviewed the results in 111 dogs with single extrahepatic CPS that were treated with AC.⁴⁶ Perioperative mortality was only 7%, but persistence of portosystemic shunting was documented in 17.5% of dogs at 8 weeks after surgery using trans-rectal technetium scintigraphy.⁴⁶ AC have also been utilized for treatment of intrahepatic CPS.^{18,24,47} In one study employing a jugular venograft technique, extrahepatic portocaval shunts were created, which allowed complete ligation of the intrahepatic CPS while facilitating AC placement on the newly created shunt.¹⁸ Nine of 10 dogs in that report survived surgery, although 5 of those experienced postoperative complications.¹⁸ Five of 8 dogs continued to have portosystemic shunting 8 to 10 weeks following surgery, and in 4 of those 5 that were further examined, evidence of MEH portosystemic shunts was found.

Results of these early clinical and experimental reports suggested that some of the original assumptions regarding the mechanism of vascular occlusion produced by the AC were incorrect. First, a number of investigators have noted that venous occlusion occurred prior to physical closure of the ameroid constrictor lumen.^{9,35,48,49} Adin et al demonstrated that the lumen of the AC decreases by only 32% after 6 weeks following implantation in the peritoneal cavity of rats, suggesting that implant associated inflammation and fibrosis, rather than hygroscopic expansion of the casein was primarily responsible for vascular occlusion produced by the AC.³⁹ Second, recent studies have suggested that there is significant variability in the rate of venous occlusion produced by the AC.^{9,10,35,48} Vascular occlusion may be dependent on several factors, including vessel diameter, AC size, implantation site, and the individual patient's inflammatory response to the AC. The variable nature of the inflammatory response to the AC in individual animals may be the cause of inconsistency in the reported venous occlusion rates, with times to complete occlusion in different models ranging from 6 to 210 days.^{9,35,48}

The occurrence of MEH shunts in a number of animals after application of the AC has led to suspicion that the AC may cause vascular occlusion too rapidly, resulting in a state of subclinical portal hypertension and MEH shunts.^{9,18} Post-operative development of MEH portosystemic shunts has been reported in 40% of dogs with intrahepatic CPS¹⁸ and 17% of dogs with extrahepatic CPS⁹ treated with AC. Data obtained in a recent experimental study in which ACs were applied to the iliac veins of dogs actually suggested that ACs do not cause a gradual decrease in venous blood flow in this model.⁴⁸ Rather, delayed acute thrombosis occurred in 3 of 6 veins within 6 days following

application. This phenomenon of acute thrombosis may explain the development of ascites and MEH shunts in dogs after application of ACs to CPS.^{9,18,46} A device that would produce a more gradual venous occlusion may avoid subclinical portal hypertension and produce complete occlusion without the formation of MEH shunts.

The AC has proven to be extremely beneficial in the treatment of animals with single extrahepatic CPS. Use of the AC has been shown to decrease overall surgery time, as well as the overall cost of performing CPS surgery when compared to conventional ligation procedures.^{24,50} Application of ACs negates the need to measure portal pressures during surgery, external manipulation is not required after surgery, and surgery is limited to one anesthetic event. Use of the AC has also been associated with a decreased intraoperative and postoperative complication rate, especially when compared to partial ligation techniques.^{46,50} Disadvantages of AC use, however, include increased dissection necessary for application, difficulty in passing the bulky AC around thin-walled shunting vessels, variable rates of vascular occlusion, difficulty in using the AC for intrahepatic shunts, development of MEH shunts, and the potential for the AC to kink the shunting vessel once in place and cause acute portal hypertension.

Cellophane

Extravascular placement of cellophane bands produces a chronic foreign body reaction similar to that described with the application of ACs, and is thought to be responsible for the progressive vascular occlusion produced by this technique.³⁵ Similar to silk and the AC, this inflammatory-mediated method of vascular narrowing would be expected to cause inter-patient variability. Factors including the initial internal diameter of the cellophane band, the initial degree of vessel attenuation, the width of the cellophane band, and the patient's natural inflammatory response may all contribute to

the degree and rate at which occlusion occurs. When the effects of cellophane banding were examined in a canine femoral vein model, it was found that the bands produced progressive, but not complete, vascular occlusion in 5 of 6 veins.³⁵ Maximal changes were seen after 14 days, and were attributed to acute inflammation.

Historically, the use of cellophane to induce fibrosis and gradual vascular occlusion has been described to study experimentally induced portal hypertension, esophageal varices, and coarctation of the aorta.^{51,52,53} Breznock first suggested the use of cellophane for treatment of CPS in 1979;⁵⁴ however, it wasn't until 1990 that Harrari et al first reported its use in a dog with a naturally occurring CPS.¹⁰ In that case, a cellophane band was placed around a portoazygous CPS, initially attenuating the shunt vessel diameter by approximately 50%. Clinical signs resolved and complete occlusion of the shunt vessel was confirmed five weeks postoperatively using mesenteric portography. More recently, Youmans and Hunt have investigated the use of cellophane in a series of clinical and experimental studies.^{29,35} In a study in which cellophane bands were used in 11 dogs with extrahepatic CPS, clinical improvement was noted in all dogs, and complete occlusion was suspected in 10 of dogs based on the results of ultrasonography and liver function tests.²⁹ Shunts were all initially attenuated to an internal diameter of 2.5 or 3 mm. Variable closure rates suggested that 3 mm may be the maximum internal diameter that would progress to complete occlusion.²⁹ Cellophane bands have also been used with variable success in dogs with intrahepatic CPS.^{55,56} In one report, banding of intrahepatic CPS in two Irish wolfhounds to an internal diameter of 6 mm and 8 mm was performed.⁵⁶ One dog was euthanized postoperatively due to the development of seizures which were refractory to treatment. Clinical signs resolved in the second dog,

though nuclear scintigraphy performed 6 months after surgery suggested that complete occlusion was not achieved.⁵⁶

It appears that the cellophane band produces slower occlusion than does the AC, although when used with an internal diameter greater than 3 mm, its ability to produce complete occlusion is questionable.³⁵ In vessels greater than 3 mm, application of the cellophane band may only produce partial attenuation. Dissection prior to application of cellophane bands is less extensive than that required for placement of ACs.²⁹ The bands are also less likely to produce mechanical distortion, or kinking of the shunting vessels as is the AC.²⁹

Advantages of cellophane banding in its application to the treatment of CPS include its ease of application, low cost, and ready availability. A single surgery is required, intraoperative portal pressure measurements are not necessary, and no external manipulation is needed after surgery. Unfortunately, variable rates of vascular occlusion and questionable efficacy on large vessels may limit its application in some clinical cases.

Thrombogenic Coils

Transvascular embolization involves the placement of thrombogenic material into a vessel lumen via catheter access and guide wires. Occlusion of the vessel lumen may occur as a thrombus develops on and around the embolizing materials.¹² Coils are constructed from a flexible metallic strip (stainless steel or platinum) and multiple polyester fibers that can be deployed through a catheter to stimulate thrombosis (Figure 2).



Figure 2. Photograph of two intravascular thrombogenic coils.

Use of thrombogenic coils (TC) for acute arterial occlusion in the treatment of arterial aneurysm^{57,58} and patent ductus arteriosus^{59,60} is well established. Advances in technology and interventional radiology have also stimulated an interest in the use of TC for gradual venous occlusion. Initial attempts at transvenous coil embolization of intrahepatic CPS were described in at least 3 individual case reports, 2 canine^{11,12} and 1 feline.⁶¹ All animals were clinically improved, although complications including coil migration and the need for multiple procedures were noted. Two¹¹ and 4¹² separate embolization procedures failed to produce complete shunt occlusion in either of the dogs. The single cat was treated successfully, although complete occlusion was demonstrated after only 48 hours following embolization.⁶¹ A subsequent case series involving 7 dogs with extrahepatic CPS and 3 with intrahepatic CPS revealed other problems associated with the use of TC.⁶² Although clinical improvement was noted in all 3 dogs with intrahepatic, and in 4 of 7 dogs with extrahepatic CPS, 3 dogs died due to migration of the coils or acute portal hypertension and other serious complications such as coil migration, severe hemolysis, and development of MEH occurred in the surviving dogs.

Complete occlusion, as determined by nuclear scintigraphy, was not evident in any of the surviving dogs. Based on this study, coil migration was found to be a major problem, particularly in the treatment of extrahepatic shunts. The authors hypothesized that this may be due the lack of supporting liver parenchyma around the shunt vessel.⁶² Results of an experimental study in which TC were placed in the femoral veins of dogs further questioned the efficacy of this technique at producing gradual venous occlusion.³⁵ In this study, TC produced complete occlusion between 3 and 7 days in only 50% (4 of 8) of canine femoral veins. Recanalization occurred shortly thereafter in 3 of the 4 (75%).³⁵

Recently the TC technique has been modified to address problems with coil migration. Two recent manuscripts describe the deployment of a caval wall stent at the junction of the shunt with the vena cava, preventing the coils placed in the shunt from migrating out of the shunt vessel.^{63,64} Using this technique shunt attenuation was achieved in 13 of 14 dogs with intrahepatic CPS, and 46% (6/13) of the dogs were ultimately deemed clinically normal.⁶⁴ Unfortunately, multiple interventions were typically required to achieve staged occlusion of the CPS.⁶⁴

Use of TC in the treatment of CPS has also been described in a small number of human patients.^{5,65} Although CPS is diagnosed infrequently in people and limited clinical data is available, it has been suggested that transcatheter embolization is preferable to surgical ligation or resection.⁵

Potential advantages of transvenous coil embolization include a shorter anesthetic period, a less invasive procedure, faster recovery and shorter hospital stay.¹² Disadvantages include the possibility of coil migration, the need for specialized instrumentation and training, multiple anesthetic episodes, the inability to obtain tissue

samples for histopathological evaluation, the inability to visualize and subjectively evaluate the abdominal contents during shunt attenuation, and the potential for rapid thrombosis and development of portal hypertension.^{12,35,61}

Clearly, initial experiences with TC have generated mixed results. While a number of dogs have improved clinically following treatment, multiple complications have also been documented. Unfortunately, the ability to gradually produce complete occlusion often requires multiple anesthetic episodes and embolization procedures, and the potential for rapid thrombosis and complete occlusion may limit TC therapy to those animals that are amenable to complete occlusion at the time of surgery. In such cases, as determined by pre and post-occlusion portal pressure measurements, TC may prove to be successful.⁶¹

Hydraulic Occluders

Hydraulic occluders consist of an inflatable silicone membrane within a polyester-reinforced, stretch-resistant cuff (Figure 3). The HO is placed around a vessel and the ring is closed by placing suture material through holes molded at each end of the cuff. Inflation of the HO can be controlled percutaneously through injections of fluid into a subcutaneous injection port that is attached to the balloon via a length of actuating tubing.

A variety of fluids have been used to fill the HO balloon, including normal saline,⁶⁶ hypertonic saline,⁶⁷ water,⁶⁸ radiographic contrast solutions,⁶⁹ indocyanine green dye,⁷⁰ and dextrose solution.⁷¹ Despite the ability of some solutions to diffuse across silicone membranes, all of these solutions have been used successfully, and an optimal filling solution has yet to be established.

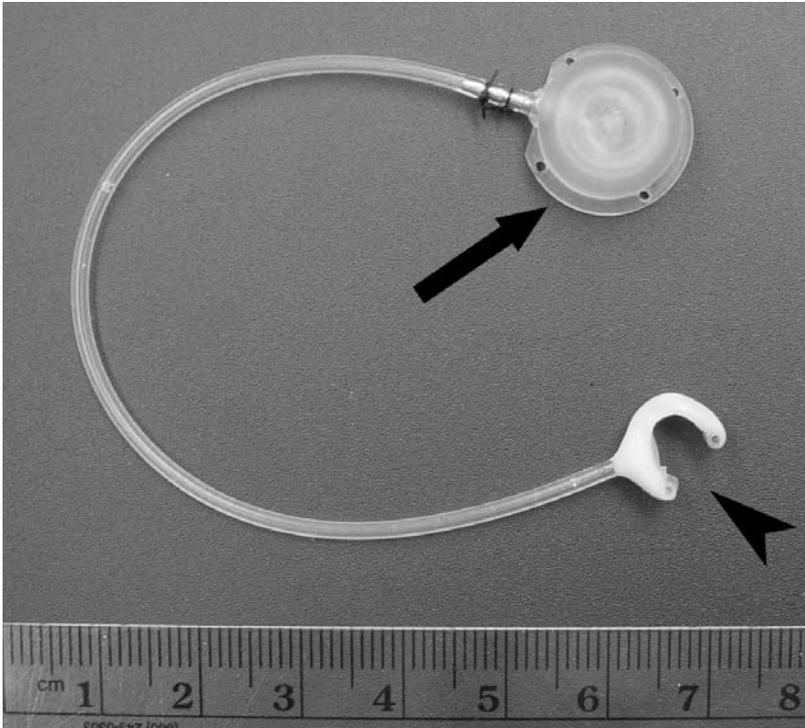


Figure 3: Photograph of a 2 mm internal diameter hydraulic occluder. Identified are the occluder (arrow head) and attached subcutaneous injection port (arrow).

Hydraulic occluders are most commonly made of medical grade silicone in order to minimize inflammation and foreign body reaction. After their introduction in the 1960's, a variety of designs were initially reported, although all were based on similar principles.^{68,72} Initially, the HO was used in acute applications to completely occlude a blood vessel, allowing calibration of electromagnetic blood flow probes to zero flow.⁶⁸ However, in 1969, Bishop and Cole reported use of the HO to maintain partial occlusion of the pulmonary artery in dogs for extended periods of time in a model of progressive pulmonic stenosis.⁷³ Subsequently, investigators have reported chronic implantation of silicone HO for up to 32 months, and documented retained adjustability up to 12 months after implantation.⁶⁶

Hydraulic occluders are used extensively in a variety of research applications, including the investigation of blood flow characteristics, organ ischemia and reperfusion, and as experimental pulmonary arterial bands.^{66,67,69,70,74,75} Similar devices have also been used clinically for more than two decades in non-vascular applications such as artificial urethral sphincters (AMS Sphincter 800 TM Urinary Control System, American Medical Systems, Minnetonka, MN), artificial anal sphincters (Acticon TM Neosphincter, American Medical Systems, Minnetonka, MN) and as adjustable gastric bands (Lap-Band, Inamed Health, Santa Barbara, CA).

Despite being used extensively in models of arterial occlusion, experience with the use of the HO for venous occlusion is limited.^{71,76,77} A recent study by Peacock et al reported the successful occlusion of the caudal vena cava in 7 dogs over a period of two weeks using a HO in a model for development of collateral circulation prior to adrenalectomy.⁷¹ The HO was easy to place surgically and manipulate postoperatively, produced and maintained complete occlusion, and was well tolerated by all dogs. Inflammation surrounding the HOs was characterized as being consistently mild.⁷¹

The HO has several theoretical advantageous attributes for the treatment of CPS including the ability to be placed at a single surgery without measurement of portal pressure, to produce gradual and total vascular occlusion, and to be reversed if necessary. Occlusion is not mediated by inflammation, but by physical compression of the vessel which may be percutaneously controlled. Cost of the HO is similar to that of the ameroid constrictor and application of the device may be performed without specialized equipment.

Use of the HO as a treatment for CPS has not yet been reported, although research investigating this application is well warranted. Prior to application of the HO in dogs with CPS, however, a study investigating the gradual occlusive abilities of the HO and resulting changes in blood flow through a venous structure is warranted.

Conclusion

Partial ligation with silk suture, ameroid constrictors, cellophane bands, intravascular thrombogenic coils, and hydraulic occluders have all been investigated as potential methods of producing gradual vascular occlusion. While all of these methods have been successful in achieving vascular occlusion, although the predictability, rate of occlusion, adjustability, and ability to achieve complete occlusion appears to vary widely amongst techniques. Although research into methods of gradual vascular occlusion has clearly improved the perioperative management of CPS in dogs, the complication rates continue to be unacceptably high, and further study is indicated.

CHAPTER 2
A PERCUTANEOUSLY CONTROLLED HYDRAULIC OCCLUDER IN A RAT
MODEL OF GRADUAL VENOUS OCCLUSION.

Introduction

Congenital portosystemic shunts (CPS) are the second most common congenital cardiovascular malformation in dogs⁷⁸, and consist of anomalous vessels that divert blood from the portal to systemic vasculature, effectively bypassing the liver.^{13,14,26} As a result, byproducts that are normally metabolized in the liver accumulate within the systemic circulation. This results in a variety of clinical abnormalities, including those associated with hepatic encephalopathy.¹⁵ Hepatotrophic substances are similarly diverted, resulting in liver hypoplasia, atrophy, and eventual hepatic failure.^{6,15}

Complete surgical ligation of CPS has been recommended to achieve resolution of clinical abnormalities and improve long-term survival,^{6,13,14,23,25,28} however, acute intra-operative complete occlusion of CPS may cause intolerable portal hypertension. In previous reports, complete ligation was achieved during a single surgical procedure in only 17% (4 of 24)¹⁹ to 55% (8 of 15)²³ of single extrahepatic CPS and 13% (15 of 114)¹⁷⁻²⁴ of intrahepatic CPS (CIPS). Some investigators have advocated staged, incremental, partial ligation to achieve complete occlusion if portal hypertension precludes complete ligation during the initial surgery.⁶ Sequential ligation requires a second anesthetic episode and surgery, resulting in increased hospitalization and increased cost. In response to these concerns, several investigators have sought to

develop a device capable of producing gradual occlusion that can be implanted during a single surgical episode.

An ideal device would be applied during a single surgical procedure, induce minimal tissue reaction, allow controlled and gradual vascular attenuation, produce eventual complete occlusion and allow reversal of attenuation should portal hypertension occur postoperatively. Clinical application of cellophane bands,¹⁰ ameroid constrictors,⁹ thrombogenic intravascular coils,^{11,12} and partial ligation with silk suture⁶⁻⁸ have been reported, though the ideal method of gradual venous occlusion has yet to be identified. In fact, experimental studies have failed to support the ability of any of these methods to produce gradual venous occlusion.^{35,48} In one study using a dog femoral vein model, cellophane, partial ligation with silk and thrombogenic coils failed to produce reliable venous occlusion during a 6-week period, while ameroid constrictors produced rapid occlusion within 14 days following surgery.³⁵ A more recent study that measured blood flow following application of ameroid constrictors to the common iliac vein in dogs demonstrated that ameroid constrictors did not actually cause gradual occlusion in this model; rather, ameroid constrictors caused delayed acute thrombosis at 5-6 days after application, presumably secondary to an inflammatory response.⁴⁸ In light of these findings, we have been seeking to develop a technique that will allow percutaneously-controlled gradual occlusion of CPS. The purpose of this study was to evaluate the effects of a percutaneously controlled silicone hydraulic occluder (HO) on vena caval blood flow in a rat model of gradual venous occlusion.

Materials and Methods

Animals

This study was performed with the approval of the University of Florida Institutional Animal Care and Use Committee. Ten male Sprague-Dawley rats, weighing between 350 and 374 g, were utilized.

Implants

Two-millimeter internal diameter hydraulic occluders (OC2, In Vivo Metric, Healdsburg, CA) were utilized throughout the study (Figure 3). Initially, the relationship between fluid volume injected and % occlusion for the 2 mm HO was determined. The silicone actuating tubing from the HO was cut to 12 cm, and then attached to a subcutaneous injection port (ROP-3.5, Access Technologies, Skokie, IL) using two 3-0 polypropylene sutures (Surgilene, Davis and Geck, Wayne, N.J.). The incomplete ring of a non-inflated HO was closed by tying a strand of 3-0 polypropylene suture material through the pre-existing holes (Figure 3). A 22-gauge, non-coring needle (22-ga x 3/4" Huber point needle, Access Technologies, Skokie, IL) was used for all injections to minimize damage to the silicone membrane of the injection port. Physiologic saline was injected and aspirated repeatedly to remove air bubbles from the HO. The non-inflated occluder was placed on a flat bed scanner (HP Scanjet 4470 C, Hewlett-Packard Co. Palo Alto, CA) and digital images were obtained. The occluder was then inflated incrementally with 0.01 mL injections of saline. Digital images were obtained after each 0.01 mL injection until the internal lumen of the occluder was completely obliterated by the inflated balloon. After completion of 3 trials, the internal area of the occluder lumen was traced for each of the stored digital images and luminal areas (LA) were measured

using a computer software program (Image J 1.27Z, National Institutes of Health, USA).

Percent occlusion after each injection was calculated based on the following formula:

$$[\% \text{ occlusion} = \text{LA non-inflated occluder (cm}^2) - \text{LA inflated occluder (cm}^2) \times 100] \div [\text{LA of the non-inflated occluder (cm}^2)].^{79}$$

Data were graphed for volume injected vs. % occlusion (Figure 4) so that investigators could achieve planned stages of luminal occlusion by injection of incremental volumes of fluid into the subcutaneous injection port.

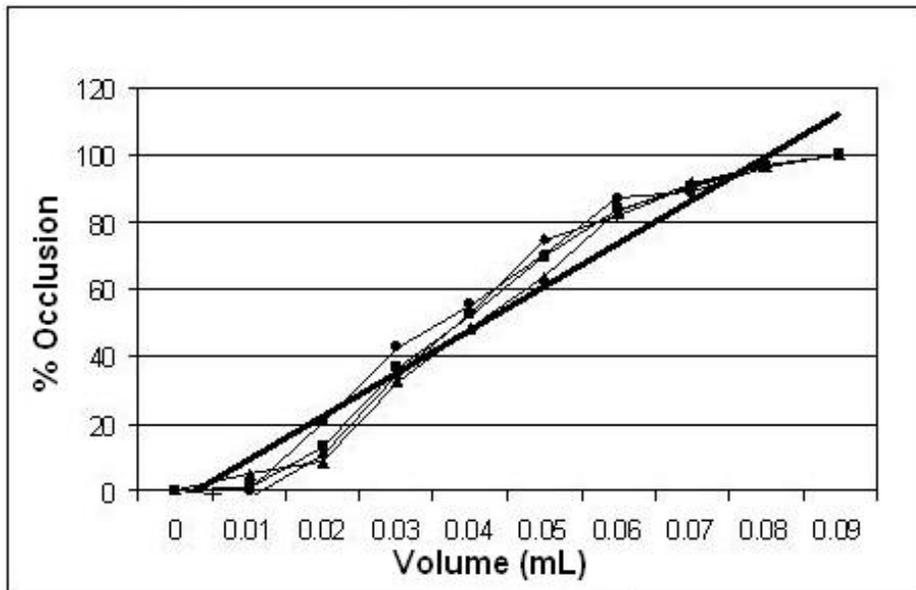


Figure 4. Graphic representation of changes in % occlusion associated with volume injected in 2 mm hydraulic occluders. Included are 3 individual trials and an overall mean, as well as a regression line (bold). Fluid was injected in 0.01 mL increments, and the corresponding occlusion of the 2 mm hydraulic occluder was measured. Volume injected was highly correlated with percentage occlusion ($R^2 = 0.9619$).

Transit time ultrasound flow probes (MC 2.5 PSS, Transonic Systems Inc., Ithaca, NY) were calibrated according to the manufacturer's directions. Briefly, the probes were submerged in a beaker of distilled water, at which point zero flow was recorded.

Surgery

Rats were anesthetized using chamber induction with 4% isoflurane (IsoFlo, Abbott Laboratories, Chicago, IL) and 100% oxygen, and were maintained on 1.5-2% isoflurane delivered via a mask for the remainder of the surgical procedure. Transit time ultrasound flow probes and HOs (OC2, In Vivo Metric, Healdsburg, CA) were sterilized with ethylene oxide prior to use. High molecular weight, isotonic sodium hyaluronate (Hylartin V, Pharmacia & Upjohn Co., Kalamazoo, MI) was selected as a filling solution to minimize diffusion of substances across the silicone membrane of the HO during long-term implantation. The actuating tubing of the HO was cut to 12 cm and filled with sodium hyaluronate using a 22-ga intravenous catheter (Intracath, Becton Dickinson, Sandy, UT) in a retrograde manner to minimize air entrapment within the HO. Actuating tubing was attached to a subcutaneous injection port using two 3-0 polypropylene sutures.

The ventral abdomen, left flank, and dorsum of each rat was shaved and aseptically prepared for surgery. The rats were then placed in dorsal recumbency on a sterile drape. A 6 cm ventral midline celiotomy was made, extending caudally from the xyphoid process. The small intestine was retracted, providing exposure of the caudal vena cava (CVC) and abdominal aorta. The right gonadal vein was ligated and transected near the CVC. Blunt dissection was used to separate and isolate the CVC from the aorta just caudal to the left renal vein. All venous branches of the CVC between the left renal vein and the iliolumbar veins were ligated and divided in an attempt to create a 2 cm segment of CVC that was free of all collateral vasculature. A flow probe was placed around the caudal aspect of isolated CVC. The HO was passed around the CVC just cranial to the flow probe, and the ring of the HO was closed using 3-0 polypropylene suture material.

The HO was then secured to the flow probe and to the hypaxial musculature with several interrupted 3-0 polypropylene sutures in an attempt to avoid kinking of the vena cava between the flow probe and the HO (Figure 5).

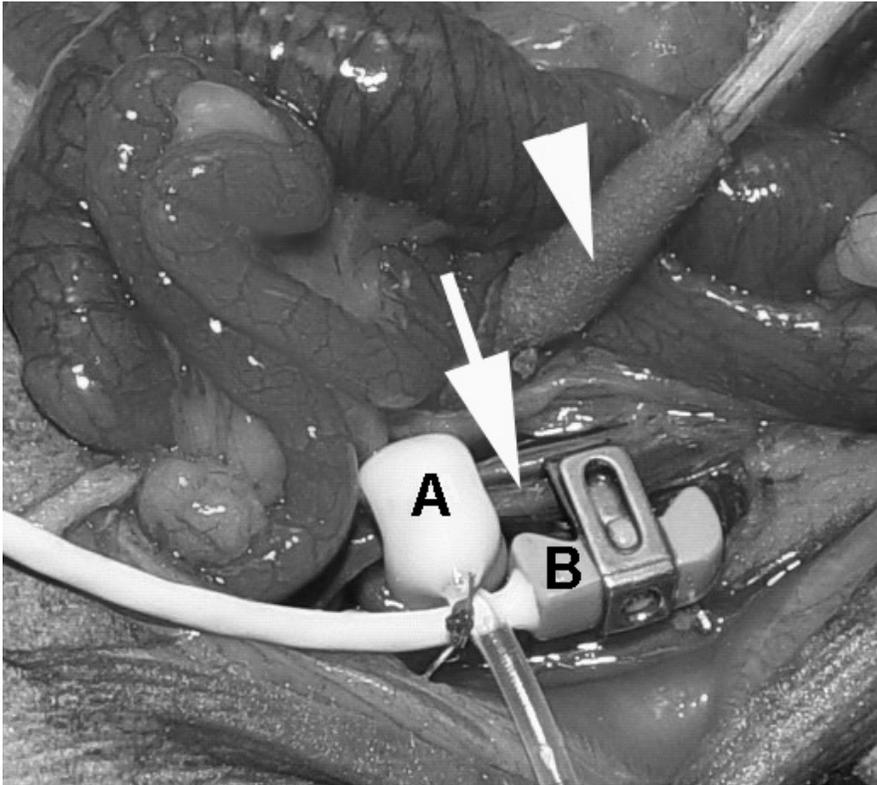


Figure 5. Intraoperative view of the instrumented caudal vena cava. A midline celiotomy has been performed and the small intestines have been reflected with the aid of a sterile cotton-tipped applicator (arrow head) to increase exposure. The hydraulic occluder (A) is located cranial to the perivascular flow probe (B) on the caudal vena cava (arrow).

After replacing the intestines into the abdominal cavity, the flow probe cable and HO actuating tubing were exited through the incision and the linea alba was closed using 4-0 polygalactin 910 (Vicryl, Ethicon Inc., Somerville, NJ) in a simple continuous suture pattern. The flow probe cable and HO actuating tubing were then tunneled subcutaneously on the left side of the abdomen and exited at the dorsal midline, just caudal to the scapulae. An injection port was attached to the actuating tubing and secured

in a subcutaneous location. The dorsal incision was closed over the injection port using several interrupted sutures, incorporating and exteriorizing the flow probe cable and connector in the process. The flow probe connector was anchored in a rigid plastic cuff (Transonic Systems Inc., Ithaca, NY), which was finally secured to the skin using several interrupted sutures. For the first 24 hours after surgery, rats were monitored every 8 hours for evidence of discomfort, during which time buprenorphine (Buprenex Injectable, Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA [0.1 mg/kg, SC, q 8-12 hrs]) was administered for analgesia.

Blood Flow Measurements

Rats were randomly assigned to either group I (n = 6 rats) or group II (n= 4). Beginning 6 to 8 days after surgery, all rats were anesthetized once weekly using the protocol described above. With the rats in left lateral recumbency, flow probe connectors were attached to a flowmeter (TS420, Transonic Systems Inc., Ithaca, N.Y.) and data acquisition system (IOX 1.6.7.9, EMKA Technologies, Falls Church, VA), allowing data from the flowmeter to be captured and analyzed on a desktop computer. Rats were allowed 1 minute to stabilize after induction of anesthesia, then automatic recording of blood flow every ten seconds for a period of three minutes was initiated. Rats in group II were weighed, and then recovered without any further intervention. Rats in group I received 6 weekly injections of 0.02 mL to cause incremental occlusion of the HO, with a final injection of 0.04 mL performed at 7 weeks after implantation to ensure complete occlusion. After injections into the subcutaneous injection ports, group I rats were weighed, and then recovered from anesthesia. Blood flow measurements recorded over the three-minute time periods were averaged and expressed as mL/min/kg body weight.

Necropsy

Eight weeks after surgery, blood flow measurements were performed as described above, and rats were euthanized while under anesthesia by intracardiac administration of pentobarbital (Beuthanasia-D, Schering-Plough Animal Health Corp., Kenilworth, N.J.). Necropsy was performed immediately and the CVC was grossly inspected for evidence of occlusion or the development of collateral circulation before being removed en-bloc with the implants. In the 2 rats with grossly patent vena cavae at the time of necropsy, catheterization of the caudal vena cava was performed and radiographs were obtained during infusion of water soluble contrast medium (Omnipaque, Amersham Health Inc., Princeton, NJ) to illustrate patency of the vessel. The flow probe was carefully removed from the CVC of all rats, and the remaining tissue sample and HO were fixed in 10% formalin for histopathology.

Histopathologic Examination

After fixation, all implanted materials and sutures were removed. Representative 5 micron sections of the vena cava were taken at the previous location of the hydraulic occluder and were routinely processed and stained with hematoxylin and eosin.

Statistical Analysis

Repeated measures analysis of variance was used to compare mean blood flow measurements over time for group I animals. Probability values ≤ 0.05 were considered significant. Post hoc analysis using a Fischer's protected least squared difference test was performed to compare weekly mean blood flow measurements to baseline values in group I animals.

Results

Volume Injected vs. % Occlusion

Percent occlusion of the 2 mm HO was highly correlated with the volume of saline injected into the infusion port ($R^2=0.9619$, Figure 4). Complete occlusion of the HO was achieved after injecting 0.09 mL of fluid in all three trials.

Surgery

Surgical placement of the implants was technically difficult due to the small size of the rat CVC and the relatively large, heavy implants. The presence of the flow probe and HO caused kinking of the vena cava at the time of surgery in several rats, despite the placement of multiple tacking sutures between the occluder, the flow probe, and the hypaxial musculature. All 10 rats survived the surgical procedure and eight-week postoperative period and showed no evidence of major postoperative complications.

Weekly Blood Flow Measurements

Signal quality from the flow probe was adequate to obtain readings in all rats at all times. Blood flow could not be detected at the 1-week time period in 1 group I and 2 group II rats. Occlusion of the CVC secondary to kinking of the vessel between the flow probe and HO was confirmed by necropsy examination in each of these 3 rats. Thus, these 3 rats were removed from subsequent analysis of mean blood flow measurements. The total volume of sodium hyaluronate required to produce occlusion (0 blood flow) was 0.06 mL (0.02 mL for 3 weeks) for one rat in group I. The remaining 4 rats required 0.17 mL (0.02 mL for 6 weeks, followed by 0.05 mL at 7 weeks). This exceeded the initially predicted volume of 0.09 mL as determined during the acute inflation of the 2 mm HO prior to the experiment (Figure 4). Weekly blood flow measurements for individual group I rats are represented in figure 6A. Zero blood flow was detected at 8

weeks in 4 of the 5 rats in group I. The residual flow recorded in the remaining rat was later discovered to be due to the presence of a collateral vessel entering the vena cava between the HO and flow probe. Mean blood flow for group I rats ($n = 5$) decreased significantly over time from 40.71 (± 24.32) mL/kg/min at day 1 to 4.68 (± 8.41) mL/kg/min at 8 weeks (Table 1, $P = 0.0094$, Power = 0.91). Post hoc analysis using a Fischer's protected least squares difference test was performed and demonstrated a significant decrease in group I mean blood flow from baseline at 4, 5, 6, 7, and 8 weeks ($P = 0.0352, 0.0342, 0.0434, 0.0050, \text{ and } 0.0001$, respectively). Both group II rats maintained blood flow at all times during the study period (Figure 6B).

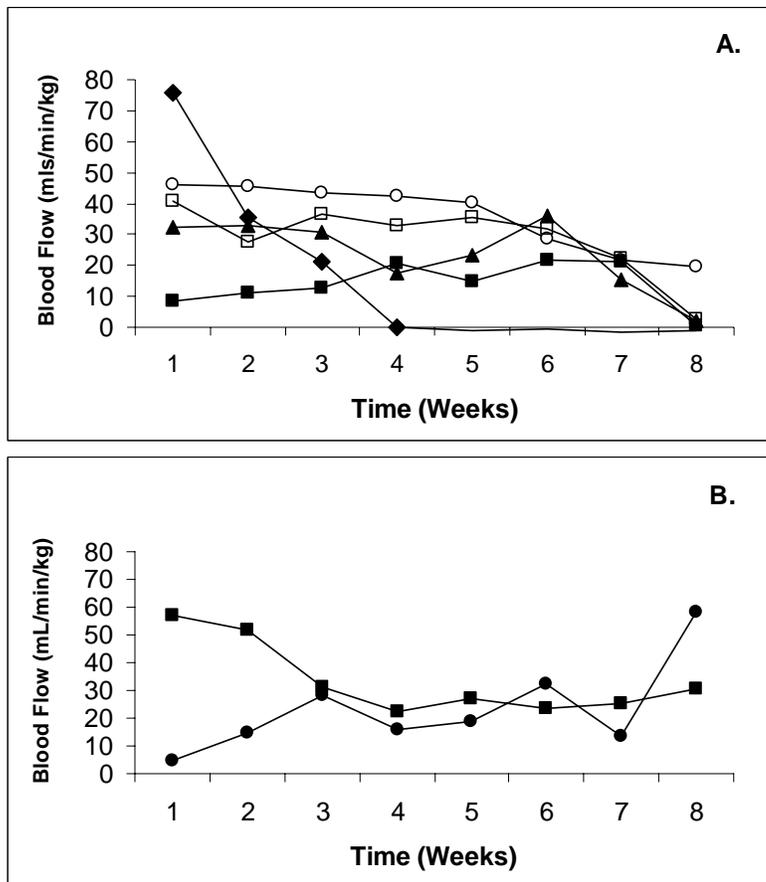


Figure 6. Individual weekly vena caval blood flow measurements. Measurements are normalized to mL/min/kg body weight. A) Group I rats ($n=5$). B) Group II rats ($n=2$).

Table 1. Summary of weekly mean blood flow for group I rats.*

	Mean Blood Flow (mL/kg/min)	Standard Deviation	Standard Error	Post Hoc Analysis (P-Value)
Week 1	40.705	24.316	10.875	---
Week 2	30.431	12.737	5.696	0.2172
Week 3	28.949	12.235	5.472	0.1596
Week 4	22.697	16.194	7.242	*0.0352
Week 5	22.582	16.737	7.485	*0.0342
Week 6	23.486	14.262	6.378	*0.0434
Week 7	15.881	10.119	4.561	*0.0050
Week 8	4.680	8.411	3.762	*0.0001

*Weekly mean \pm SD and SE vena caval blood flow measurements are normalized to mL/min/kg body weight and are depicted for group I (n = 5 rats) over the 8-week study period. Blood flow decreased significantly over time (P = 0.0094, Power = 0.91) during gradual inflation of the hydraulic occluder. Significant changes (denoted by asterisks) from baseline blood flow were noted at 4, 5, 6, 7, and 8 weeks (P = 0.0352, 0.0342, 0.0434, 0.0050, and 0.0001, respectively).

Necropsy

Necropsy examination revealed occlusion of the vena cava secondary to kinking between the flow probe and the vena cava in 1 group I and 2 group II rats. Complete occlusion of the CVC at the HO site was confirmed in the 5 rats from group I. Collateral vascular development caudal to the flow probe, consisting of multiple small vessels between the CVC and ilio-lumbar veins, was identified in all group I rats. A small vessel was identified entering the vena cava between the FP and HO in 1 group I rat, allowing blood to pass through the FP and bypass the HO. The CVC remained patent in two group II rats, as documented via contrast radiography (Figure 5). Adhesions between the omentum and the HO were present in all cases.

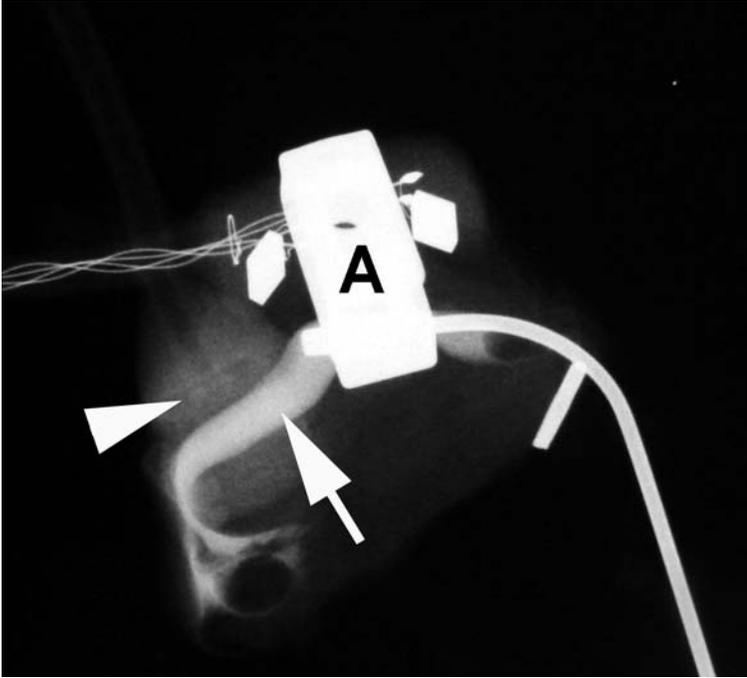


Figure 7. Radiographic image documenting caval patency. The image was obtained after injection of 0.5 mls of water soluble contrast medium (arrow) into the caudal vena cava of a rat in group II, demonstrating patency at the level of the hydraulic occluder (arrow head) 8-weeks after surgery. The perivascular flow probe (A) is seen caudal to the hydraulic occluder.

Histopathologic Findings

Sections of vena cava had varying degrees of fibrosis throughout the vessel wall and contained organizing thrombi leading to variable degrees of occlusion. Areas of mural fibrosis were infiltrated by small numbers of lymphocytes, plasma cells and neutrophils. The negative spaces left by the removed materials was surrounded by dense bands of fibrous connective tissue with an internal layer of small to moderate numbers of lymphocytes, plasma cells, macrophages and fewer neutrophils and eosinophils. A proliferation of small vessels surrounded the areas of fibrosis in some sections. Fragments of suture material were surrounded by granulomatous to pyogranulomatous inflammation.

Discussion

To the authors' knowledge this is the first study to successfully document a gradual decrease in venous blood flow after application of an occlusive device. Previous investigators have used contrast radiography,^{9,29} clinical signs,⁶ portal scintigraphy,⁹ and serum biochemical indices^{9,29} to confirm effective occlusion of CPS. Although these methods are effective in confirming functional occlusion of CPS, they are unable to characterize whether vascular occlusion was gradual or acute. In fact, aside from the current manuscript, only 1 other study has been performed using direct measurement of venous blood flow after application of a device intended to produce gradual vascular occlusion.⁴⁸ Interestingly, the results of that study have called into question the ability of the AC to produce gradual venous occlusion, showing instead that delayed, acute occlusion occurred only 6 days after application of an ameroid constrictor to the common iliac veins in normal dogs.⁴⁸ The clinical implications of this information are substantial, suggesting that the methods used for evaluation of vascular occlusive devices may require further evaluation and that surgical techniques that have been validated using indirect methods may not actually produce the gradual vascular occlusion that would be advantageous for the treatment of dogs with CPS.^{9,29}

The hydraulic occluder used in the study consists of three components: an inflatable hydraulic balloon, a variable length of actuating tubing, and a subcutaneously placed injection port. The entire HO is made of silicone (Silastic Q7-4849 Biomedical Grade LSR), making it suitable for long-term implantation. Silicone is widely accepted as a non-inflammatory, biocompatible polymer and has been used extensively in surgical implants for several decades.⁸⁰ The tissue response to silicone implants is similar to that seen with other foreign bodies, although minimal in comparison.⁸⁰ A fibrous tissue layer

is typically formed, encasing the implant.⁸⁰ The long-term implantation of the hydraulic occluder and the flow probe on the caudal vena cava of rats in group II did not result in a decrease in blood flow during the eight week study period, suggesting that the alterations in blood flow among group I rats was not due to a chronic inflammatory response, but was instead directly related to physical compression of the vessel after inflation of the HO. Despite acute occlusion associated with the combined use of the HO and FP which occurred in 3 rats in the current study, we did not observe complications that could be ascribed solely to the HO. Considering the fact that silicone implants are routinely used in human patients with limited adverse sequelae, we feel that the HO would be suitable for long-term implantation in dogs with CPS

There was an appreciable variation between individuals with respect to baseline blood flow using this model. Baseline blood flow was not compared statistically between groups due to the low numbers of group II rats with available data. When data from the individual rats is evaluated (Figure 6A and B), it is apparent that the widest variation in flow rates occurs during the first 3 measurements, whereas blood flow seemed to stabilize after 3 or 4 weeks. The authors attribute this variation is due to perioperative factors such as inflammation produced by vessel manipulation or poor early coupling between the CVC and FP. Unfortunately, the reasons for these variations cannot be determined from the data obtained through this study.

A variety of fluids have been used to fill HOs during acute and chronic studies, including normal saline,⁶⁶ hypertonic saline,⁶⁷ water,⁶⁸ radiographic contrast solutions,⁶⁹ indocyanine green dye,⁷⁰ and dextrose solution.⁷¹ Manufacturer recommendations at the time of this study indicated that high molecular weight substances would be preferred for

maintenance of chronic inflation with minimal diffusion out of the silicone HO. Based on this recommendation, sodium hyaluronate (Hylartin V, Pharmacia Animal Health) was empirically chosen as a filling solution due to its high molecular weight, iso-osmolarity, and safety in the instance of accidental leakage into the peritoneal cavity.⁸¹ The product is also readily available as a sterile preparation, making it suitable for use in body cavities. The authors' experience in using the HOs suggested that *in vivo* filling volumes required to produce occlusion after chronic implantation may exceed those required for acute occlusion, *in vitro*. Our goal in this study was to produce slow occlusion in greater than 4, but less than 8 weeks. As a result, we elected to use filling volumes determined in the *in vitro* study as a starting point for an injection protocol that should not have produced complete occlusion in less than 5 of the 0.02 mL injections. Weekly injections of 0.02 mL were performed until 7 weeks, at which time a 0.04 mL injection was performed to ensure complete occlusion. The total volume of sodium hyaluronate ultimately required to produce vascular occlusion after chronic implantation was greater than that required to produce acute occlusion of the HO in 4 group I rats. This may be explained by several different mechanisms, including diffusion of air, mechanical creep experienced by the inflated silicone occluder, and HO size variations secondary to lack of standardization during manufacturing. Despite attempts to eliminate the introduction of air into the HO at the time of initial filling, the small size of the 2 mm HO and the viscous nature of the sodium hyaluronate often resulted in the entrapment of a small air bubble with the cuff of the HO. Unfortunately, silicone is gas permeable and it is likely that partial deflation of the HO occurred as air bubbles trapped within the HO balloon were displaced. Due to the fact that very small filling volumes for the 2 mm HO used in

this study were extremely small (0.09 mL), diffusion of small volumes of trapped air may have led to significant deflation of the occluder. In the larger HOs with increased volume capacities that would be required for use in the treatment of CPS in dogs, the inclusion of small air bubbles at the initial filling would be less likely to ultimately affect occlusion to the same degree. Another contributing factor may be related to creep, which is defined as plastic deformation of a material after the application of mechanical stress.⁸² If creep occurred over the 8-week study period, the silicone used in construction of the HO may have expanded slightly, creating a larger potential filling volume than those recorded during acute inflation prior to the study. Lastly, the injection volume to % occlusion curve was acquired after performing three consecutive filling trials with the same HO. Subsequent gross examination of the 10 HOs used during this study revealed obvious differences in size. This lack of standardization is likely due to the manufacturing process, which is performed by hand and is therefore subject to some variability. If so, the predicted filling volume may have been slightly different between individual HOs.

Perivascular flow probes have been utilized in both acute and chronic applications and have proven to be accurate and reliable.^{83,84} Volumetric flow is measured by emitting a plane wave of ultrasound through the vessel of interest.⁸⁴ A loose fit around the vessel is acceptable, thereby making the flow probe ideal for use on easily collapsible vessels such as veins. It has been shown that the presence of the flow probe around venous structures in chronic applications does not appear to affect venous circulation or hemodynamics when the device is used alone.^{48,84} However, the adjacent location of the HO and the large flow probe on the rat CVC in the current study caused an obvious

tendency to kink the vessel between the large implants, predisposing the vessel to thrombosis.

Advantages of the rat model used in this study included low cost, genetic similarity of animals, ease of maintenance and use of a mammal that is low on the phylogenetic scale. The size disparity between the rat CVC and the required instrumentation may, unfortunately, limit the use of this model in future studies. Although the interior diameter of the HO and flow probe were appropriate for the size of the vessel, the weight and exterior diameter of the implants were poorly adapted for use on the thin walled, collapsible CVC. Despite attempts to stabilize the CVC, flow probe, and HO to the surrounding musculature with several interrupted sutures, the size and close proximity of the implants caused kinking of the vessel segment postoperatively in three of the rats. These three rats had no flow detected at any time point during the 8-week study period. Use of smaller implants or a larger animal model may eliminate similar complications in subsequent studies. Alternatively, the HO could be evaluated without the presence of a flow probe, though this would preclude the non-invasive blood flow monitoring that was required to document gradual venous occlusion in this experiment. Despite these limitations, results of the current study demonstrated a gradual decrease in venous blood flow over an 8-week study period, with complete occlusion achieved in all rats.

CHAPTER 3
MECHANICAL AND PHYSICAL PROPERTIES OF SILICONE HYDRAULIC
OCCLUDERS

Introduction

Hydraulic occluders (HOs), consist of an inflatable silicone membrane within a polyester-reinforced, stretch-resistant cuff. Inflation of the HO can be controlled percutaneously by injection of a filling solution into a subcutaneous injection port that is attached to the occluder via a length of actuating tubing (Figure 3). Chronic implantation of silicone HOs has been described in experimental models since the 1960's.⁷³ More recently, investigators have reported the potential for various clinical applications of the HO in dogs, including use of the HO as an artificial urethral sphincter,⁷⁹ or as a means of producing gradual venous occlusion for the surgical treatment of adrenal neoplasia.⁷¹

Despite extensive experimental use, there is a paucity of information regarding the reliability of silicone HOs during chronic implantation. Earlier experiences with the HOs, (Chapter 1) have raised concerns regarding potential size differences among HOs secondary to manufacturing. Manufacturing variability may negatively impact the ability to accurately predict filling volumes and adjustments of the level of occlusion. In addition, silicone is known to act as a semi-permeable membrane⁸⁵⁻⁹⁰ and the diffusion properties of various filling solutions may affect long-term maintenance of occlusion. The purpose of this study was to examine size differences among HOs of the same model, diffusion of various filling solutions including: saline, sodium hyaluronate, and air

from the HO, and to describe changes in internal pressure over time in three different models of the HO that were obtained from a single manufacturer.

Materials and Methods

Evaluation of Size Differences Among HOs

Five each of 2, 5, and 20 mm internal lumen diameter HOs (HO2, HO5 and HO20, respectively) were obtained from a single manufacturer (DOCXS Biomedical Products and Accessories, Ukiah, CA). The incomplete ring of each non-inflated HO was closed by tying a strand of 3-0 polypropylene suture material (Surgilene, Davis and Geck, Wayne, N.J.) through the pre-existing holes molded at either end of the HO cuff. HOs were all prepared in a similar fashion, in which actuating tubing was cut to a standard length of 6 cm. HOs were filled in a retrograde manner using a 22 ga. jugular catheter in an attempt to eliminate air from within the HO. Injection ports (ROPAC-3.5, Access Technologies, Skokie, IL) were also pre-filled using non-coring huber point needles (Posi-Grip Huber Point Needle, Access Technologies, Skokie, IL) to eliminate residual air prior to attachment of the HO actuating tubing. HOs were attached to injection ports by threading the actuating tubing over the male connector and securing it with two individual circumferential 3-0 polypropylene ligatures. The closed, non-inflated HOs were placed on a flat bed scanner (HP Scanjet 4470 C, Hewlett-Packard Co. Palo Alto, CA) and digital images were obtained and saved to a desktop computer for further analysis. Using a computer software program, (Image J 1.27Z, National Institutes of Health, USA) the internal luminal area (LA) of each HO was traced and calculated three times. The mean LA for each HO was then calculated and used for statistical analysis.

Diffusion of Saline and Sodium Hyaluronate Through the HO

All HOs were properly exercised prior to use, and then assembled and filled as previously described. Two HOs of each model (HO2, HO5 and HO20) were randomly assigned to be filled with either sodium hyaluronate (SH) (Hylartin V, Pharmacia and Upjohn, Kalamazoo, MI) or 0.9% sterile saline. Saline is considered to be the standard filling solution for inflatable silicone implants in human beings due to its isotonicity, ready availability, and low cost. Based on previous studies suggesting that molecular weight plays a major role in diffusion of substances through the silicone membrane of the HO,⁸⁹ we elected to compare saline to SH: an isotonic, non-toxic, high molecular weight substance. HOs were filled with additional saline or SH through their injection ports until complete occlusion was noted. The inflated HOs were scanned to document 100% occlusion and weighed to within 10^{-3} g using a laboratory scale. (APX-203, Denver Instrument, Arvada, CO) HOs were then placed in a bath of simulated body fluid (SBF) kept at a constant 37° C.⁹¹ SBF was replaced on a weekly basis. HOs were removed from the SBF once daily for the first 29 days, then once weekly for a total of 8 weeks to be dried, scanned, and weighed. The digital images were analyzed using a computer software program as described above to determine the internal LA of the HO. These values were then compared to the initial, non-inflated LA for each HO in order to calculate the % occlusion using the following formula: [% occlusion = LA non-inflated occluder (cm²) – LA inflated occluder (cm²) x 100] ÷ [LA of the non-inflated occluder (cm²)].⁷⁹

Diffusion of Air Through the HO

Two each of HO2, HO5, and HO20 were utilized. All HOs were properly exercised prior to use. HOs were all assembled and filled as previously described. A

huber point needle was used to fill each of the HOs with air until complete occlusion was noted. The inflated HOs were scanned to document 100% occlusion. HOs were submerged into a bath of SBF kept at a constant temperature of 37° C. HOs were removed from the SBF every 4 hours and digital images were obtained for calculations of LA as previously described.

Changes in Internal Pressure Over Time

Internal pressures were measured over a 30-day study period in the HO2, HO5, and HO20 occluder models (1 of each model). All HOs were properly exercised prior to use. Each occluder was prepared as shown in Figure 8. Actuating tubing was secured to the injection port, T-port (T-5S-CS-UF, Access Technologies, Skokie, IL), and brass fitting with 3-0 polypropylene ligatures and additional silicone (Silastic, Dow Corning Corporation, Midland, MI) to prevent leakage at the tube insertion point. The HO, injection port, actuating tubing, and brass fitting were filled with 0.9% saline in a retrograde manner, taking care to eliminate air entrapped within the closed system. The entire apparatus was attached to a pressure transducer (PX 603-030G5V, Omega Engineering Inc., Stamford, CT) and process meter (DP25 B-E-A, Omega Engineering Inc., Stamford, CT), which were in turn connected to a desktop computer for data storage and analysis. Once assembled, HOs were filled via the injection port with additional 0.9% saline until complete occlusion was achieved. HOs were scanned at this time to document 100% occlusion. HOs, including the injection port and all actuating tubing were submerged into a bath of SBF that was kept at a constant temperature of 37° C and changed weekly.⁹¹ Pressure monitoring was initiated when HOs were completely occluded and submerged in the SBF. Software that allowed 24 hour pressure monitoring

(IOX 1.6.7.9, EMKA Technologies, Falls Church, VA) was employed to record internal pressure on an hourly basis for a period of 30 days.

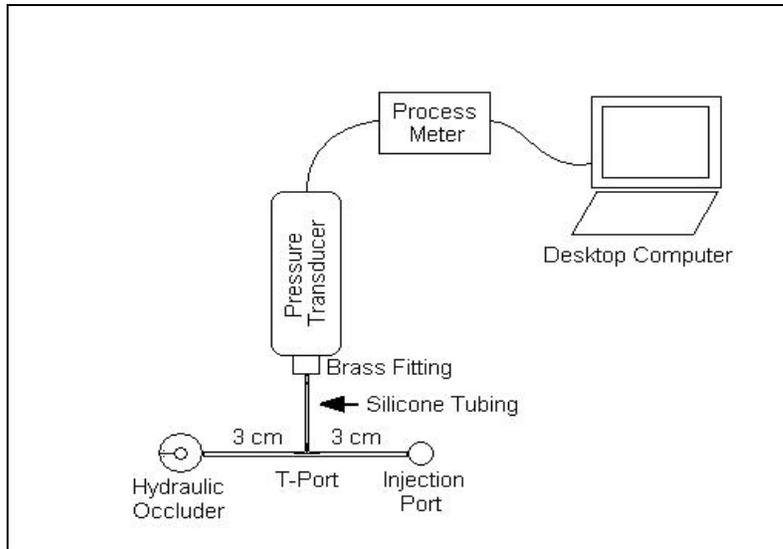


Figure 8. Schematic diagram representing the experimental setup used for evaluating changes in internal pressure over time within individual hydraulic occluders.

Statistical Analysis

Differences in luminal area between individual HOs were compared using an ANOVA. A 2 way repeated measures ANOVA was used to evaluate differences in weight and % occlusion over time and between different filling solutions. $P < 0.05$ was considered significant. A Bonferroni/Dunn post-hoc test was used to test for interactions when appropriate.

Results

Evaluation of Size Differences Among HOs

Mean closed, non-inflated LA values ranged from 0.038 cm^2 to 0.053 cm^2 for the HO2, 0.156 cm^2 to 0.189 cm^2 for the HO5, and 2.227 cm^2 to 2.855 cm^2 for the HO20. Mean LA values, along with their standard deviations for the 5 HOs of each size are

displayed graphically in figure 9. Statistical analysis revealed a significant difference in the non-inflated internal LA among the 5 HOs in all three sizes tested ($P < 0.001$).

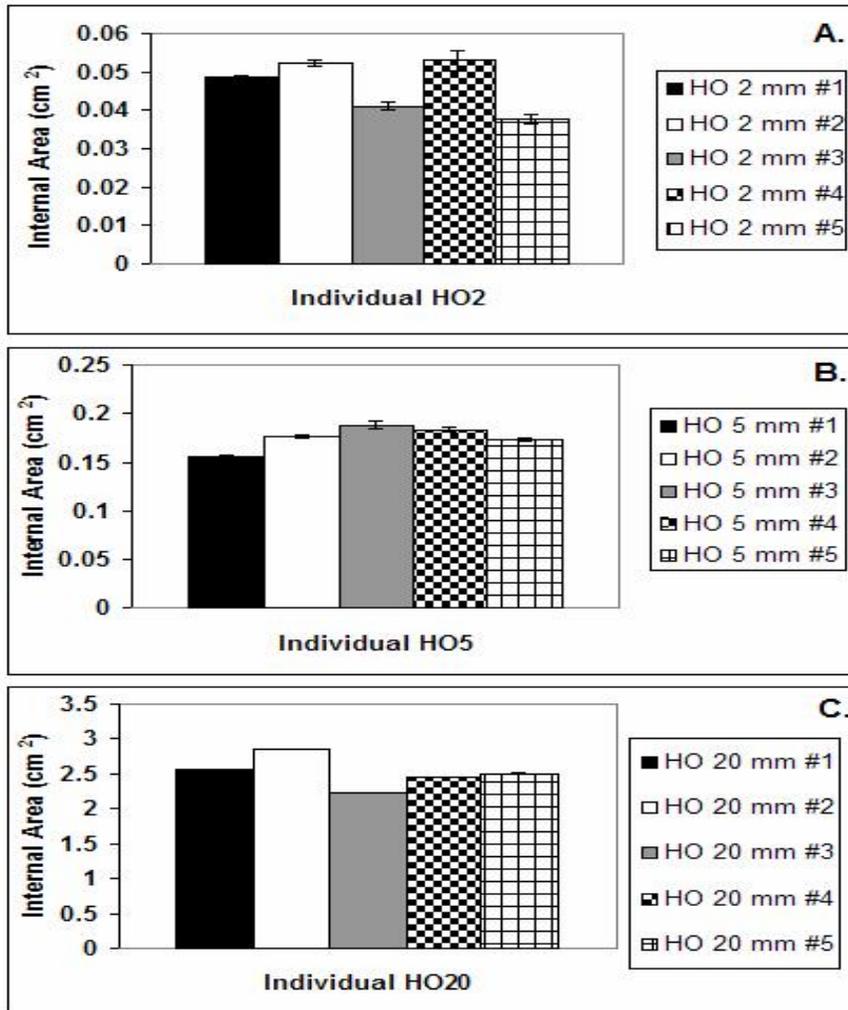


Figure 9. Graphic representation of the variations in luminal area within 3 models of the non-inflated hydraulic occluder.

Diffusion of Saline and Sodium Hyaluronate Through the HO

Statistically significant decrease in weight was seen in all HOs over time ($P < 0.0001$). However, no significant difference was found when comparing weight changes in HOs filled with saline to those filled with SH. (HO2: $P = 0.7650$, Power = 0.055; HO5: $P = 0.5340$, Power = 0.075; HO20: $P = 0.4742$, Power = 0.085). Weight loss over time is represented graphically in figure 10.

A statistically significant loss of occlusion was seen in all HOs over the 64-day study period ($P < 0.0001$), however loss of occlusion was much more dramatic in HO2 (mean 26.6%) compared to HO5 (mean 4.5%) and HO20 (mean 0.4%), regardless of which filling solution was used. No significant difference was found when comparing the change in % occlusion in HOs filled with saline to those filled with SH. (HO2: $P = 0.2704$, Power = 0.150; HO5: $P = 0.3960$, Power 0.102; HO20: $P = 0.2650$, Power = 0.153). Change in % occlusion over time is represented graphically in figure 11.

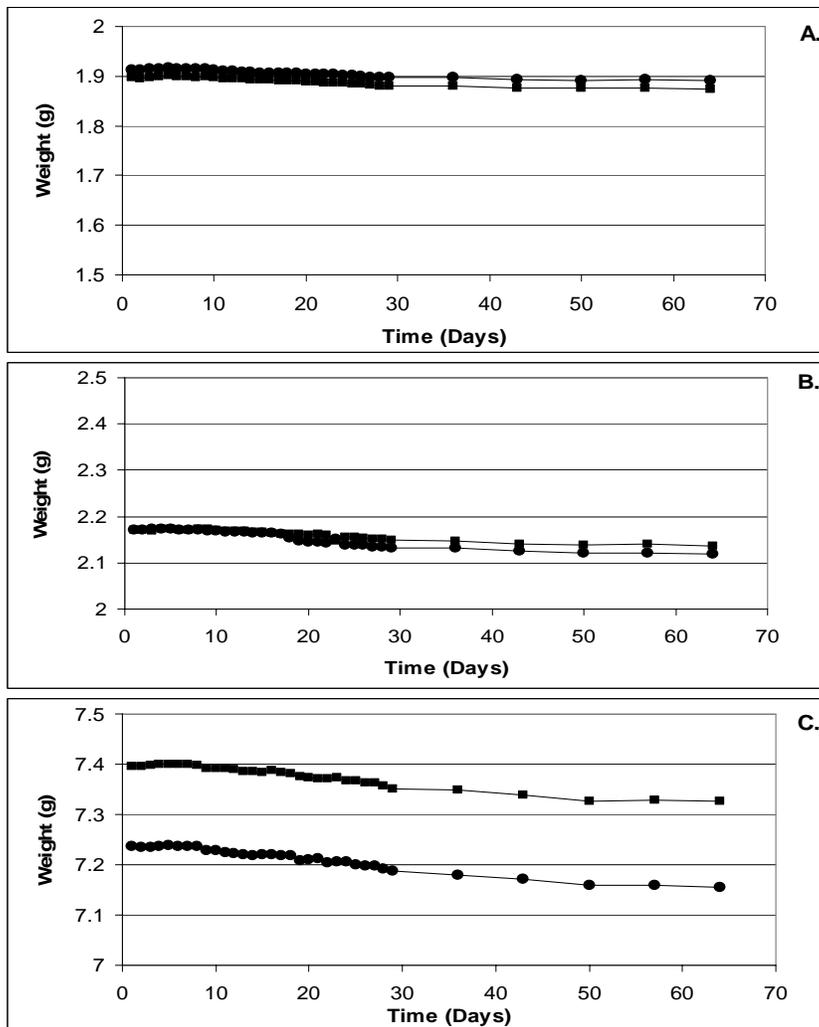


Figure 10. Graphic representation of the mean weight loss over time for 2 mm (A), 5 mm (B), and 20 mm (C) hydraulic occluders filled with either saline (●) or sodium hyaluronate (■).

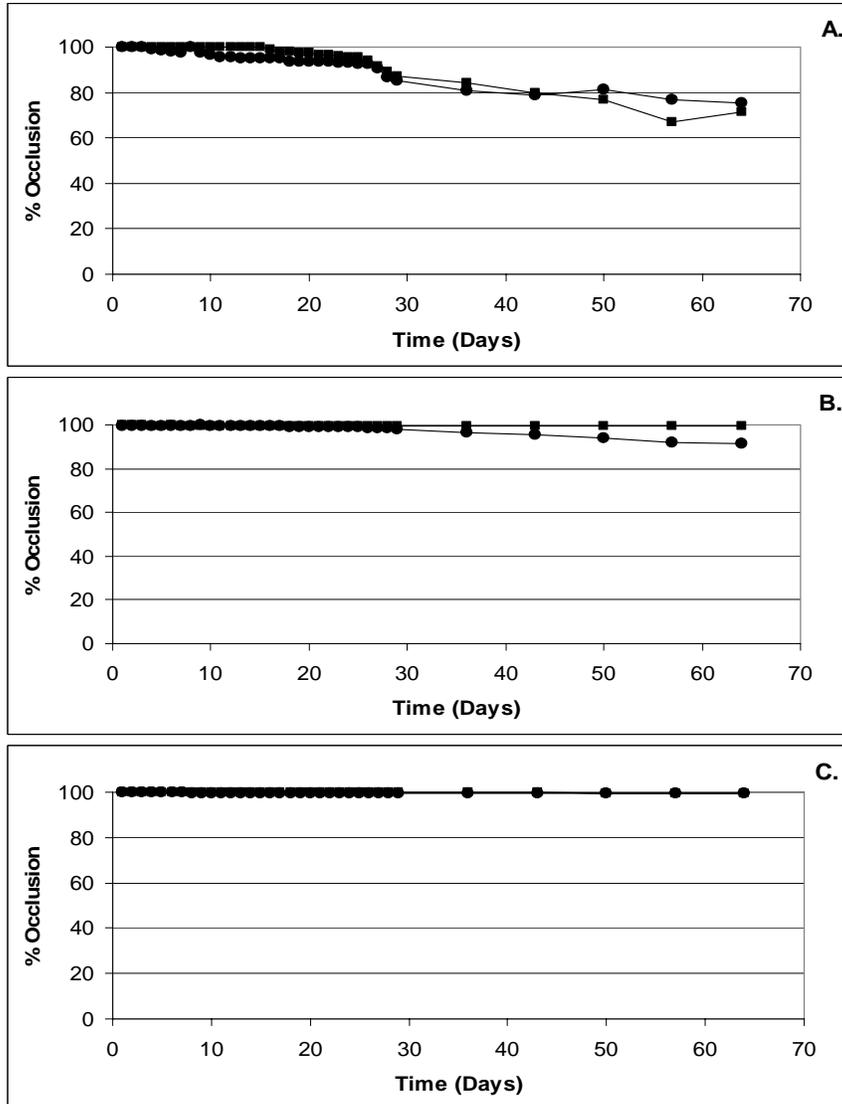


Figure 11. Graphic representation of the mean % occlusion over time for 2 mm (A), 5 mm (B), and 20 mm (C) hydraulic occluders filled with either saline (●) or sodium hyaluronate (■).

Diffusion of Air Through the HO

A significant loss of occlusion was seen in all air-filled HOs over a twelve-hour period ($P < 0.0001$) (Figure 12). A significant interaction was also noted between change in occlusion over time and HO size ($P = 0.0014$), with a more rapid loss of occlusion occurring in the smaller HOs.

Changes in Internal Pressure Over Time

Pressure changes over time for HO2, HO5, and HO20 are displayed graphically in figure 13. The smaller HOs generated a greater internal pressure at filling, and experienced a more rapid loss of pressure than the larger HOs. All HOs experienced the most significant pressure losses within the first 5 days, and tended to plateau as time progressed. Despite losses in pressure, complete occlusion was documented in both the HO2 and HO20 at the end of the study using digital imaging. The HO5 had undergone a slight loss of occlusion deemed to be 3.5% using the same image analysis software described previously.

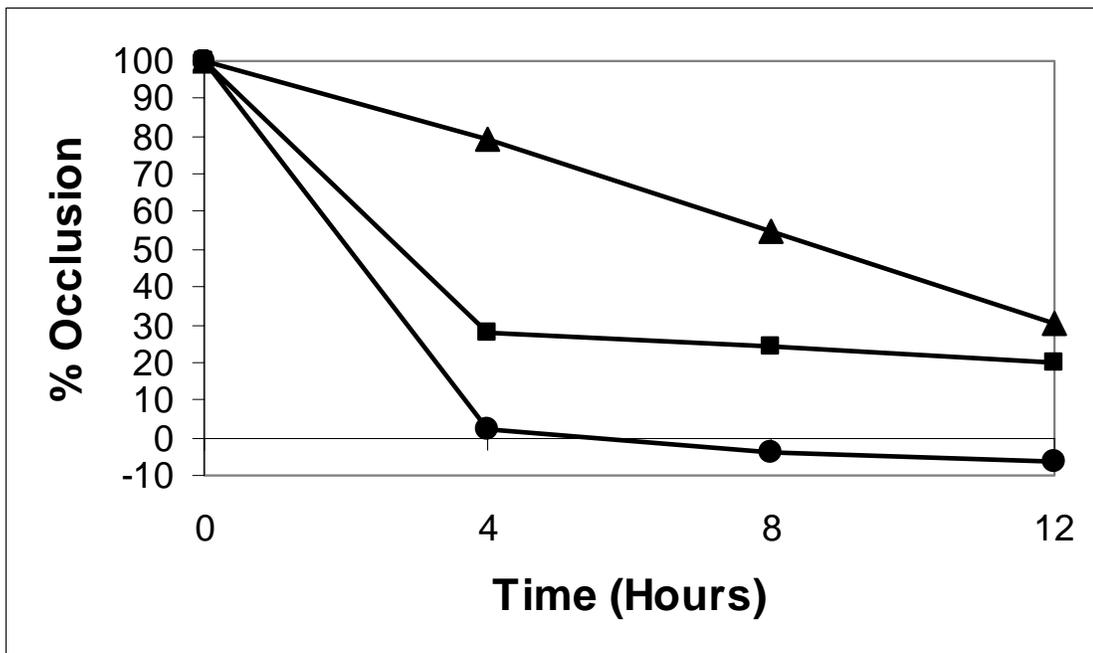


Figure 12. Graphic representation of the mean change in occlusion over a period of 12 hours for 2 mm (■), 5 mm (●), and 20 mm (▲) hydraulic occluders filled with air. Note the trend towards a more rapid loss of occlusion in the smaller HOs.

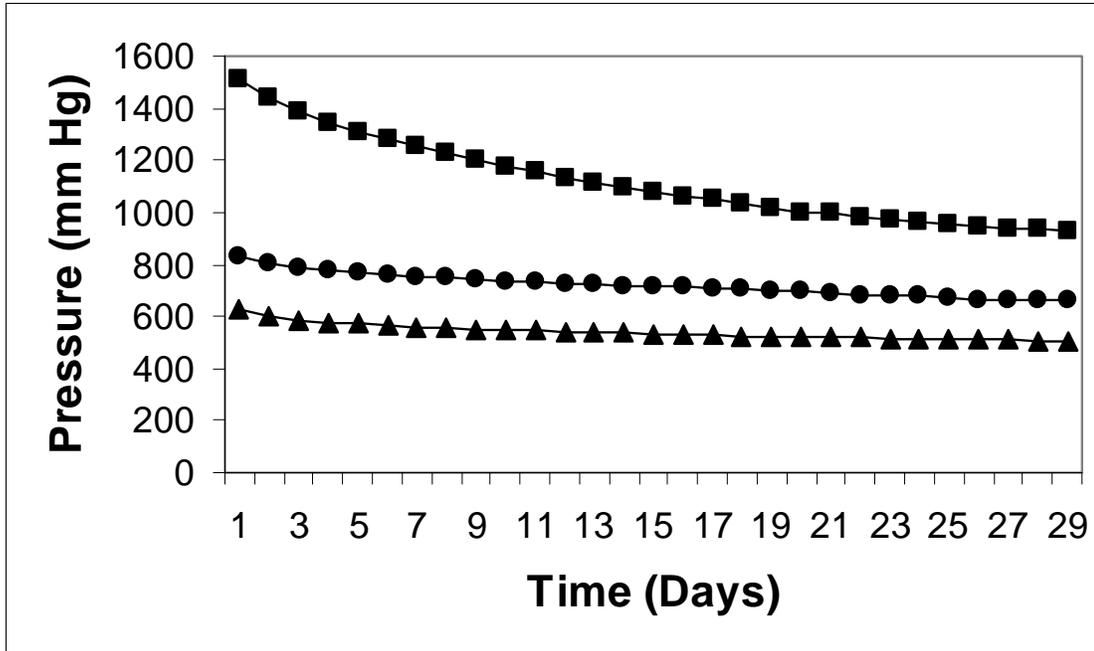


Figure 13. Graphic representation of the change in internal pressure over time for 2 mm (■), 5 mm (●), and 20 mm (▲) hydraulic occluders filled with saline.

Discussion

Previous work with the silicone HO in a model of chronic vascular occlusion (Chapter 2) demonstrated a marked disparity between the predicted filling volume and the actual volume required to produce vascular occlusion *in vivo*. Data from the current mechanical evaluation of silicone HOs has revealed a number of factors that may have contributed to variations in filling volumes during chronic applications. For example, gross observation of the HOs had suggested that there was variation in the luminal area due to inconsistencies in the manufacturing process. Hydraulic occluders are manually assembled from sheets of medical grade silicone membrane, Dacron backing and silicone tubing, using silicone gel to “hand weld” the seams. Digital images of the occluders acquired during the current study did reveal significant variations in LA between individual HOs in all three sizes that were evaluated (HO2, HO5, and HO20). The

disparity in luminal area was most remarkable for the HO2 size, with a range in LA from 0.038 to 0.053cm², a range that encompasses nearly 40% of the total area. It is also important to note that while the occluders are sold in “sizes” that are intended to describe the internal diameter formed by the closed ring (i.e., the HO2 corresponds to a 2mm internal diameter), the luminal areas measured in our study do not correspond to the area of a circle with the diameter denoted by each occluder size. For example, a circular occluder with a 2mm internal diameter would have a calculated LA of πr^2 or 0.0314cm² while the measured LA of these occluders actually ranged from 0.038 to 0.053cm². Luminal area was measured after “exercising” the occluders as recommended by the manufacturer, which may have contributed to increasing the LA to a figure that exceeded the original LA due to acute stretching and deformation of the silicone HO. Thus, the data from our current in vitro study would suggest that there is significant manufacturing variability between individual HOs and that the measured luminal area after exercising of the HOs does not necessarily reflect the advertised luminal diameter. Variations in LA are particularly apparent in the smaller occluders (HO2) and this fact, combined with the extremely small filling volumes required for occlusion, may have a profound effect on fine control of rate of vascular occlusion when these smaller devices are applied *in vivo*.

A second concern in chronic application of silicone HOs was diffusion of filling solutions through the thin silicone membrane, leading to deflation of the occluders over time. Polydimethylsiloxane, generally referred to as silicone, is a highly cross-linked elastomer that is largely bio-inert and as such has been used extensively in medical implants since the late 1940's.^{92,93} It is a semi-permeable material, and diffusion of various solutions and solutes across silicone membranes has been well documented.⁸⁵⁻⁹⁰

Diffusion across the HO silicone membrane is likely affected by numerous factors, including hydrostatic pressure within the HO, filling solution osmolarity and molecular weight, surface area of the silicone membrane, solute lipophilicity, osmotic gradient of the environment to which the HO is exposed, temperature, and thickness of the silicone membrane. A variety of solutions have been used to fill silicone HOs in previous short-term experimental studies, including normal saline,⁶⁶ hypertonic saline,⁶⁷ water,⁶⁸ radiographic contrast solutions,⁶⁹ indocyanine green dye,⁷⁰ and dextrose solution.⁷¹ Indeed, vast experience with diffusion of filling solutions from similar silicone implants has been obtained through clinical application of artificial urethral sphincters, breast implants and tissue expanders in human beings. In these clinical applications, isotonic saline is currently the most commonly employed filling solution.⁹⁰ Although these implants are expected to behave similarly to the HO investigated in the current study, our primary concerns with deflation of the HO are related to the unique application of the device for chronic maintenance of complete vascular occlusion. As demonstrated in the current study, complete inflation of the HO leads to the generation of extremely high internal hydrostatic pressures, particularly in the smaller HOs. However, despite the elevated hydrostatic pressure gradient, minimal diffusion of either saline or SH occurred over the 8 week study period. Diffusion of solutions across a semi-permeable membrane is also related to the thickness of materials used in membrane construction. Despite the fact that there is some variation in materials used among different occluder sizes, such that the membrane and actuating tubing of the HO20 are thicker than those used in the construction of the HO2 and HO5 (0.020" compared to 0.007", respectively), diffusion of filling solution did not appear to differ substantially among HOs of the three sizes tested.

In addition, no statistically significant difference was noted when comparing weight loss or loss of occlusion between saline filled HOs and those filled with SH. Although it is possible that a significant difference in diffusion would be detected between filling solutions if a larger number of occluders was used in each experimental group, the similarity of results in the current study would suggest that a clinically significant difference between saline and SH would be unlikely.

In order to fully evaluate the results of our diffusion study, we must also analyze the accuracy of the model. The *in vitro* model used in our study was based on the recommendations of a previous group performing a similar evaluation of filling solutions in silicone implants.⁸⁹ In an attempt to simulate the intra-abdominal environment, implants were submerged in SBF kept at a constant 37°C to simulate the temperature, pH, and ion concentrations found in plasma.^{89,91} Implants were weighed and the non-inflated LA was determined prior to submersion in the SBF to provide a baseline. Both weight and LA determination proved to be highly accurate and reproducible means by which serial measurements could be obtained, as reflected by the repeatability of the measurements and small significant differences that both provided.⁹⁰ Weight loss, as well as loss of occlusion occurred to some degree in all HOs, suggesting that diffusion of filling solutions occurs over time. Initial decreases in HO weight preceded any visible loss of occlusion. This may be explained by the fact that weight change was a more sensitive test, and that small volumes of filling solution may have been lost before it was grossly evident as a loss of occlusion. It is also possible that while filling the HOs to complete occlusion, they were inadvertently over-inflated. In this situation, any excess filling solution could initially diffuse out of the HO without affecting occlusion.

A third concern regarding deflation of HOs over time was the effect of air bubbles that may be entrapped within the silicone balloon during retrograde filling with fluid solutions. Permeation of air and other gases through silicone membranes has been documented previously and far exceeds that of fluid filling solutions.⁸⁷ Data obtained in the current study confirms the rapid nature of air diffusion from the HOs, which led to a marked loss of occlusion in air-filled occluders over a matter of hours (Figure 12). Based on this data, we suspect that entrapped air bubbles may have contributed to variability in filling volumes required to reach complete occlusion in our previous use of the HO. Based on the rapid nature of air diffusion through the silicone membranes, it is recommended that investigators attempt to remove any air bubbles from the HO by filling the actuating tubing in a retrograde manner using a long catheter as described. Small air bubbles would likely be more significant in the HO2 compared to the HO5 and HO20 due to the smaller initial filling volume.

As a final aspect of this study, we evaluated changes in internal pressure of the HOs over time. Internal hydrostatic pressures after complete inflation of the HOs were inversely proportional to the size of the HO; a phenomenon that is likely related to the total surface area of the occluders. An initial rapid decline in pressures occurred over the first 5 days, followed by a more gradual rate of decline that continued for the entire 28-day study period for all three sizes of HO. The initial rapid decrease in internal pressures is likely to be associated with creep (plastic deformation over time) experienced by the silicone material after application of tensile stress. Polymers are known to undergo permanent deformation when subjected to chronic stress.⁸² It is possible that stretching

of the HO occurred over time, leading to an increase in the potential filling volume of the HO, a resulting decrease in internal pressure, and ultimately a partial loss of occlusion.

This study has documented that inconsistencies in manufacturing, diffusion of fluid or gaseous filling solutions through the silicone membrane and mechanical creep may all affect the reliability of silicone HOs used in chronic applications. Although measurable loss of occlusion and diffusion of filling solutions occurred in all HOs during the 64-day period, these changes were most substantial in smaller HOs (HO2 and HO5). The clinical implications of changes in occlusion of the larger HO20 occluders would likely be negligible. Detectable differences were not demonstrated between saline and the higher molecular weight SH with regard to loss of occlusion or diffusion of filling solutions over time, and either substance would serve as an acceptable filling solution for long-term maintenance of occlusion.

CHAPTER 4 CONCLUSION

Despite successful treatment of congenital portosystemic shunts (CPS) via partial occlusion with silk suture, the application of ameroid constrictors and cellophane bands, as well as the deployment of intravascular thrombogenic coils, an ideal surgical device has yet to be identified. An ideal device for CPS therapy would allow its safe application to the shunting vessel, complete non-invasive adjustability in the postoperative period, a predictable and adjustable rate of vascular occlusion and the capability of attaining complete occlusion. Although its application in treating congenital portosystemic shunts has not yet been described, the silicone hydraulic occluder (HO) theoretically has many of the qualities listed above. Unfortunately, there is a paucity of information present in the scientific and medical literature regarding use of the HO at producing gradual and complete venous attenuation in long term applications.

Prior to use of the HO for treatment of CPS in canine patients, it was felt that an in vivo study should be performed describing the HO's ability to gradually produce and maintain complete venous occlusion. In the rat study described in Chapter 2 of this manuscript, it was shown that the HO was indeed capable of producing a gradual decrease, and eventual complete cessation of blood flow through the caudal vena cava of rats. However, several complications with the model were identified, as well as some potential issues that may affect future use of the HO in a clinical setting. Most notably, the ability to accurately correlate the level of occlusion with the injected filling volume appeared to be somewhat inconsistent in vivo. Potential causes for these discrepancies

were thought to include size differences among the HOs secondary to the manufacturing process, the diffusion of air and/or filling solution from the HO over time, and the phenomenon common to many elastomers known as creep (defined as plastic deformation over time).

In Chapter 3 of this manuscript, a series of in vitro studies are described that were designed to further evaluate the factors mentioned above. It was found that there is a statistically significant difference in the size of uninflated HOs in each of the 3 size categories evaluated (2mm, 5mm, and 20mm). It was also evident that all HOs undergo a slight loss of occlusion over time, regardless of whether or not saline or sodium hyaluronate was used as a filling solution. This loss of occlusion was much more apparent in the 2mm HOs, and was almost insignificant in the 5 mm and 20 mm HOs. Air diffused very rapidly from the HOs in all cases, although again this was more apparent in the 2mm HOs. All HOs experienced a loss of internal pressure over time, implying that creep may have occurred.

HOs are an appropriate means of producing controlled, gradual, and complete venous occlusion. The ability to adjust and maintain occlusion over time appears to be more difficult in the small (2mm) HOs, and may be related to a combination of factors including: (A) inconsistencies in the manufacturing process which complicate extrapolating volume injected : % occlusion curves from one occluder to another, (B) the unavoidable entrapment of air upon initial filling and its subsequent displacement from a small potential filling volume, (C) diffusion of filling solution from within the occluder, and (D) the physical changes that are associated with creep and occur within the HO over time. Larger HOs, despite being subject to the same factors, do not appear to be as

severely affected, and are much more capable of maintaining occlusion over an extended period of time.

Future studies examining the use of HOs in the treatment of CPS are warranted. Use of HOs with sizes ranging from 5 mm to 20 mm would be expected (corresponding to reported CPS vessel diameters), and as such would be less susceptible to the complications noted with use of the 2 mm HOs. Repeating the experiments described in Chapter 3 is also warranted in attempt to generate improved power of the statistical analyses by increasing the number of experimental subjects.

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BIOGRAPHICAL SKETCH

Colin W. Sereda began his post-graduate education in a two year pre-veterinary program through the Department of Agriculture at the University of Alberta in Edmonton, Alberta, Canada, between 1995 and 1997. He was then accepted to the Western College of Veterinary Medicine at the University of Saskatchewan in Saskatoon, Saskatchewan, Canada, where he completed a 4 year program in veterinary medicine and graduated in 2001 with a Doctor of Veterinary Medicine. He then completed a clinical internship in small animal medicine and surgery at the Virginia-Maryland Regional College of Veterinary Medicine in Blacksburg, Virginia, between 2001 and 2002. After completing the internship, Colin moved to Gainesville, Florida, in 2002, where he entered the Master of Science degree program at the University of Florida's College of Veterinary Medicine. In combination with the master's degree, Colin is also completing a residency in small animal surgery, and is scheduled to be done in the summer of 2006.