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I am submitting herewith a thesis written by Tommy Lee Washington entitled “Novel Design of a Portosystemic Shunt Occluder.” I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Polymer Engineering.

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Novel Design of a Portosystemic Shunt Occluder

A Thesis
Presented for the
Masters of Science
Degree
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Tommy Lee Washington
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ABSTRACT

In some breeds of dog, specifically the Yorkshire Terrier, a genetic defect exists that causes a hepatic portosystemic shunt to form. A hepatic portosystemic shunt is a vessel that when present cause blood to bypass the liver, which is responsible for filtering waste from the digestive system and moving it out of the body. An implantable polymeric device was constructed that would slowly occlude the hepatic portosystemic shunt and force the blood to stop flowing through the bypassing vessel and flow through the vessel to the liver over 3-4 months time.

The portosystemic shunt occluder device consist of three polyoxymethylene components, a swelling hydrogel (poly (acrylic acid)), and a degrading copolymer – (Poly (lactide-co-caprolactone)). The poly (acrylic acid) was used to move the piston component of the device upward to constrict the portosystemic shunt placed inside the device. Eight milligrams of poly (acrylic acid) had a swelling ratio of 20.10 ± 1.63 , a swelling rate of 0.071 mm/s^2 , and generated 25mN of force. Eight milli-Newtons is the amount of force that is produced by blood flowing in a four milli-meter diameter vessel.

The degradation properties of two compositions of poly (lactide-co-caprolactone) (40 percent lactide and 60 percent caprolactone; 86 percent lactide and 14 percent caprolactone) were analyzed and relationships were developed that would aid in determining the degradation rate of the copolymers in respect to the copolymer composition and the environmental

temperature. The poly (lactide-co-caprolactone) controlled the rate that the piston moved by providing a counter force to the poly (acrylic acid). The poly (lactide-co-caprolactone) (40:60), when loaded into the device, degraded in 86 days in a phosphate buffered saline solution at a temperature of 37°C.

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CHAPTER 1

PORTOSYSTEMIC SHUNTS

Background

The hepatic portal system is the system by which the blood flows through veins that begin as capillary beds in the stomach, intestines, and spleen. As food passes through the stomach and the intestines, Figure 1, certain nutrients are absorbed from it into the portal system. Products excreted for digestion, such as bile acids, and produced by digestion, such as ammonia, can also be reabsorbed into the portal system as well. But as the nutrients are absorbed, the organs of the system also produce waste such as bile acids, ammonia, urea, and lipids. This waste is then deposited back into the blood.

Blood constituents absorbed by the organs of the digestive system are filtered by the liver. In a normally functioning system, blood that is processed by the liver is devoid of the waste. In some dogs such as the Yorkshire terrier, a small breed of canine, they may lack the

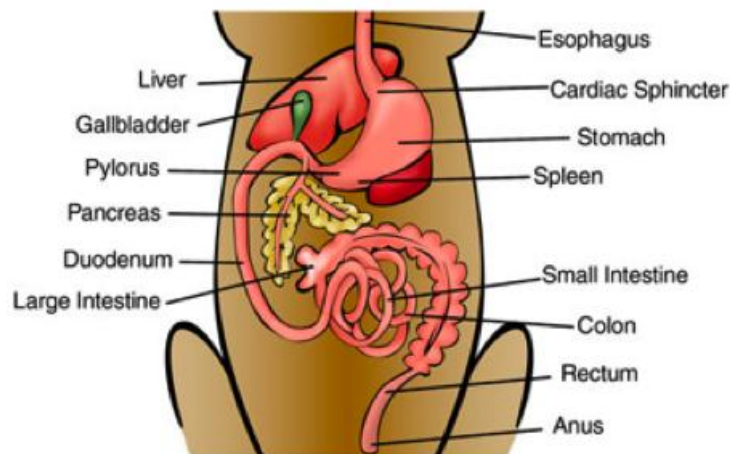


Figure 1: Canine Digestive System [2]

proper function of the liver at birth.¹ The impaired liver therefore makes removal of toxins difficult.

Function of the Liver

The structure of the liver can be simply described as two inputs and one output. The blood in the circulatory system enters the liver by way of two sources: the hepatic portal vein, which transports oxygen deprived and nutrient rich blood, and the hepatic artery, which transports oxygen rich blood, Figures 2 and 3. The portal vein is the main blood source to the liver, Figure 2. Portal blood is derived from the capillary beds present in the stomach, intestines, and the spleen, Figure 3 and 4. The portal vein is a combination between the superior mesenteric vein, which originates from the digestive visceral, and the splenic vein, which comes from the spleen. After the portal vein enters into the liver it expands back into the capillaries, which then release the blood into the liver and then filters the excess waste from the organs of the digestive system. The filtered blood is absorbed back into the capillaries which pool to form the hepatic veins, which drain into to the inferior vena cava, which flows to the heart and re-circulates the blood, Figure 4 and 5.

Portosystemic Shunts

A portosystemic shunt is a blood vessel that allows blood to bypass the liver, move directly into the inferior vena cava and heart, thus eliminating the participation of the liver. The liver is the vital organ responsible for the body's digestion, excretion, nutrient storage, nutrient

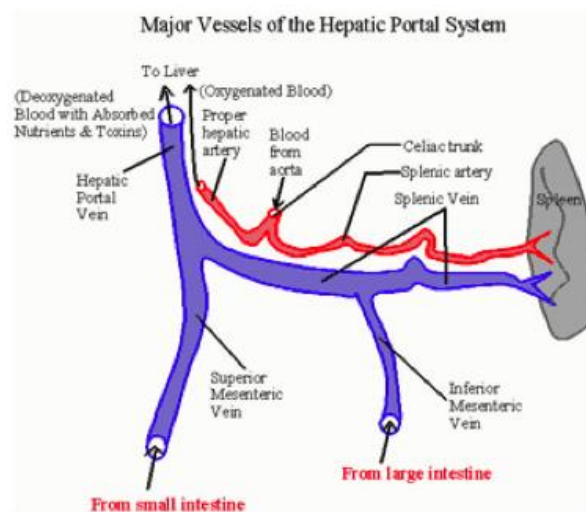


Figure 2: Major Vessels of the Hepatic Portal System [4]

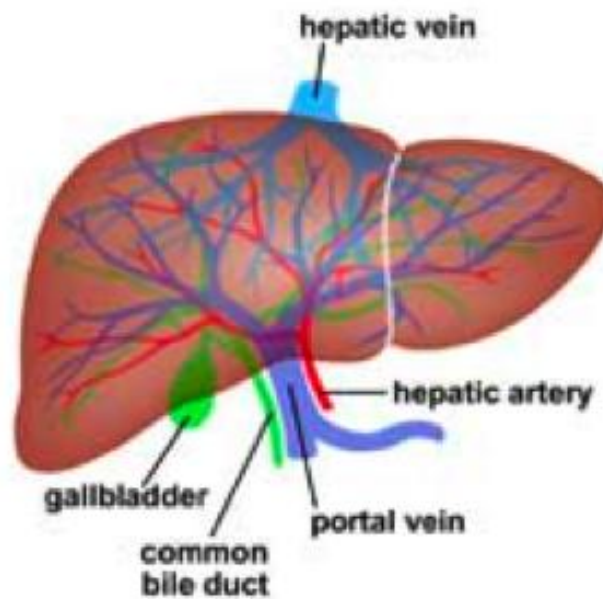


Figure 3: Structure of the Liver [5]

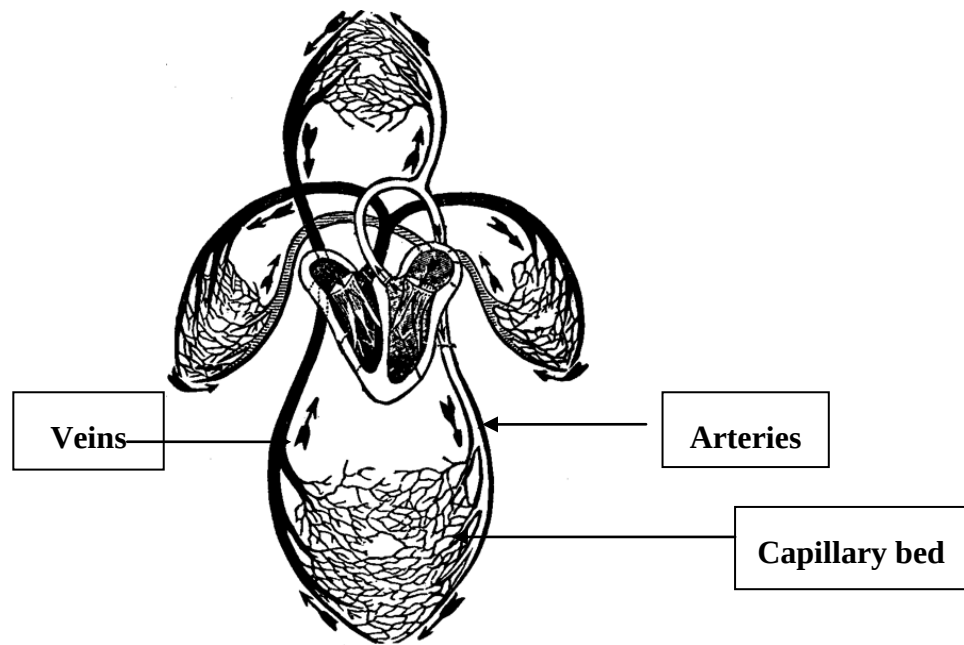


Figure 4: Canine Circulatory System [3]

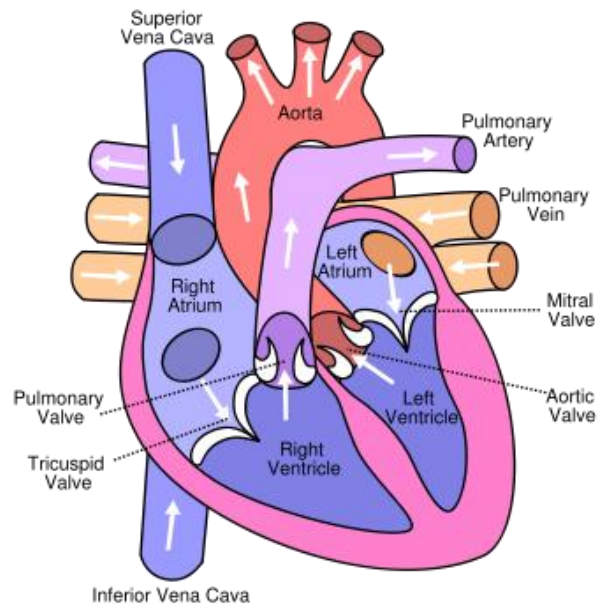


Figure 5: Canine Heart

conversion, detoxification, and synthesis of glycogen. More importantly, the constitutive cells of the liver aid in the removal and storage of glucose from the blood, as well as lipids, vitamins, copper and iron. The liver also removes elements such as ammonia and converts it into a safer chemical form of nitrogen (i.e. urea), which is used to make proteins for cell growth and repair and also creates the kidney concentration gradient for urine production. Excess ammonia is excreted from the body by way of the kidneys. The liver also acts as a filter to prevent toxins from reaching the heart that could lead to a potentially fatal condition of sepsis.

In dogs, the ductus venosus is a large portosystemic shunt that circumvents the liver during fetal development. Portosystemic shunts are classified based on three categories: method of development, structural integrity, and type of bypass. The development of portosystemic shunts is classified as either congenital or acquired. Congenital shunts are vascular abnormalities that are thought to occur with abnormal fusion or incomplete regression of embryonic abdominal vasculature during fetal development or failure of normal closure after birth.⁶ The ductus venosus in normal dogs closes functionally within days and is structurally obliterated within 3 weeks post birth.

Acquired portosystemic shunts occur as a response to improper function of the liver or liver disease. For example, cirrhosis of the liver decreases its functionality and increases the blood pressure in the portal system. Acquired shunts assist in diverting blood away from a nonfunctional liver and protect a damaged liver from further deterioration. Congenital shunts are more common than acquired shunts and have been noted to be genetically linked in breeds of

dogs such as Maltese, Irish Wolfhound, and particularly the Yorkshire Terrier. In a study conducted by Karen Tobias at the University of Tennessee College of Veterinary Medicine, a significant number of Yorkies seen in a 12 year period were diagnosed as having congenital portosystemic shunts, and that percentage has been increasing over the past 10 years. This study concluded that Yorkshire Terriers were 35.9 times more likely than other breeds to be born with portosystemic shunts. The reason for Yorkshire predisposition is attributed to the amount of inbreeding in the breed, hence why it is considered to be a hereditary disorder; but the mechanism of inheritance has yet to be elucidated.⁶

Portosystemic shunts can be classified as extrapatic or intrahepatic, single or multiple, and congenital or acquired. Structural classifications are based on the relative position of the branching off of the shunt to the portal vein from the vena cava (Figure 6). If the base of the shunt begins at the main portal vein and then flows directly to the inferior vena cava, the shunt is classified as an extrahepatic portosystemic shunt (Figure 6(b)). Typically, these shunts can achieve a diameter up to one centimeter in size. They theoretically develop from abnormal anastomoses of the vitelline veins with the cardinal vessels, which form the tributaries of the caudal vena cava and its prehepatic segment.⁶ If the base begins within the branching of the portal vein and flows to the inferior vena cava, then it is classified as an intrahepatic portosystemic shunt (Figure 6c). Intrahepatic portosystemic shunts are thought to develop from incomplete closure of the ductus venosus or retention of segments of vitelline vessels that should have degenerated.⁶

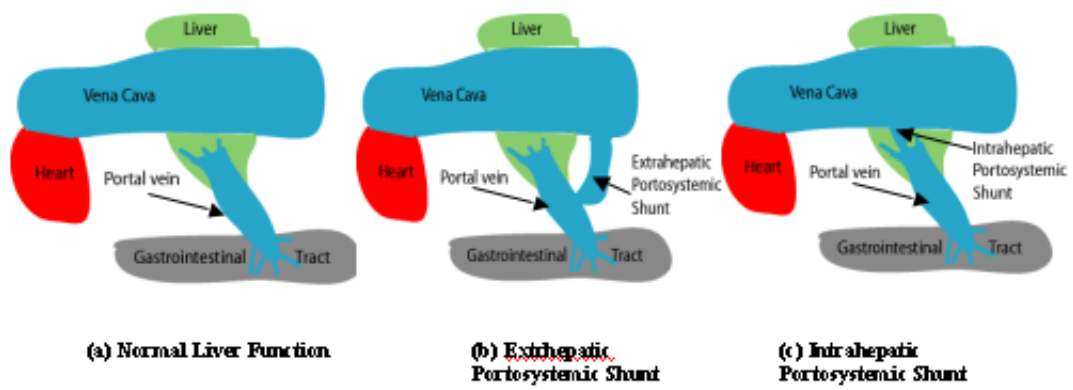


Figure 6: Types of Portosystemic Shunts

Normal canine fetal hepatic structures (Figure 6(a)) are derived from the vitelline and umbilical veins. In the fetus, fusion and degeneration of various sections of these vessels leads to the formation of the hepatic sinusoids, portal system, and the fetal ductus venosus, which are necessary for proper circulation and filtering of the blood.⁷ It should be noted that environmental and genetic factors alone or in combination are theoretically thought to be responsible for shunt development in canines, such as those which can be seen in Figure 7.

Portosystemic shunts are also classified by if there are single or multiple shunts present in the system, Figure 8. For the purposes of this research, discussion will be restricted to the most common portosystemic shunt in small canines, congenital extrahepatic single shunts.

To understand the nature of congenital portosystemic shunts, it is necessary to understand their formation. Fetal development begins with formation of the zygote. Pregnancy is divided into three periods: the germinal period, the embryonic period and the fetal period. After fertilization, the blastocytes begin separation into the three main layers: endoderm, mesoderm, and ectoderm. The circulatory system develops out of the mesoderm layer. During the germinal period, the basic structure of the mesoderm layer begins to form.

Next the embryonic period marks the stage of organ development on a broad scale. Because the organs are not fully developed, organ systems such as the circulatory system are not capable of supporting the other organ systems through their own facilitation. Thus, the other developing systems (i.e. skeletal and muscle) rely on the mother's circulatory system to provide the necessary nutrients and nourishment for growth. During this stage, shunts such as the ductus



Figure 7: Portosystemic Shunt in Canine

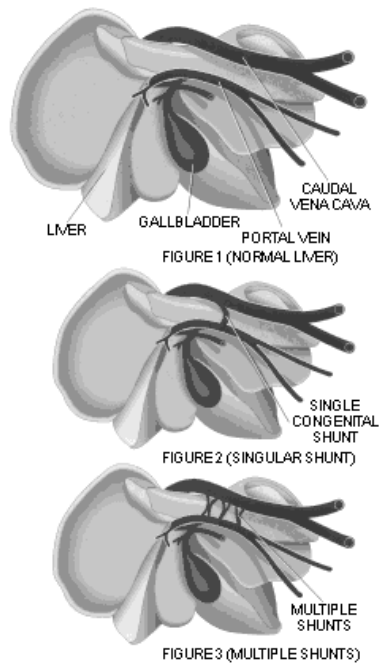


Figure 8: Single and Multiple shunt System

venosus allow the developing blood flow to bypass other developing and partially functional organs. At this point in the fetal development, the organ systems that are within the fetus cannot fully operate, and consequently, the fetus is dependent upon the mother's working organs. Shunts provide a way of directing blood away from not fully functional organs. The components of the plasma (water, electrolytes, and proteins) are transferred to the fetus from the mother via the umbilical cord, because at this stage the heart and liver of the fetus are not fully functional. The mother's liver does the work of filtering out toxins, storing sugar, and producing protein for the unborn fetus. Therefore, these shunts are necessary to avoid disturbing the developing liver of the fetus and to deliver the filtered blood nutrients to the fetus from the properly working organs of the mother.^{8,9} During the fetal period, the organs more fully develop, and the fetus becomes less dependent upon the mother's organs. After birth, these shunts close to allow the neonate to rely upon its own body functions independently of the mother. But in some situations shunt closure does not occur.

In the past, there have been a number of approaches used to occlude these extrahepatic shunts. In the early 1990's, surgical ligation was the recommended treatment for congenital portosystemic shunts.¹⁰ However, this simple technique has experienced many setbacks. Mortality rates associated with surgical ligation reached percentages as high as 21%.¹⁰ Complications due to this intervention included portal hypertension, anesthetic complications, portal vein thrombosis, or status epilepticus.¹⁰

Characteristics such as constant small stature, poor muscle development, seizures, quiet

demeanor, and behavioral abnormalities (circling, disorientation, unresponsiveness, staring into space, head pressing) are common symptoms of dogs with extrahepatic shunts present. Dogs with extrahepatic shunts normally have low blood urea nitrogen (BUN) and albumin concentrations, display microcytosis (a condition where red blood cells are smaller than normal), or acutely anemic. These blood related abnormalities permit diagnosis via blood analysis. The urine analysis is also a method for detection. The amount of liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) increases in dogs with congenital portosystemic shunts resulting in small spiky crystals (ammonium biurate) in their urine also. Though the previously mentioned symptoms/characteristics are visible in subjects with shunts present, they are not specific to the presence of a liver shunt. Veterinarians use these abnormalities as signs that a shunt could be the possible reason for the animal's poor health. The abnormal signs act as a clue indicating to test the bile acid or ammonia concentrations to evaluate the liver's function.⁹

Shunts can be treated with proper diet and medication, but only approximately one third of the dogs medically treated will meet their average life expectancy. Over half the dogs that are treated medically are euthanized within 10 months of being diagnosed because of neurologic related problems, such as seizures and behavioral changes, or progressive liver damage.⁹

Many of the toxins produced in the intestines are due to the protein intake, therefore it is important to reduce the amount of protein in the dog's diet. Food for adult dogs and puppies contain approximately 25% and 29% protein, respectively. In addition they also contain meat

byproducts. Dogs with shunts are restricted to high quality proteins made from vegetables or milk that contains 18%-22% protein or less on a dry matter basis. The diet should also be rich in antioxidants and vitamins, low in iron and copper, and easily digestible.⁹

One of the main medications that is prescribed to control the side effects of the shunt being present is Lactulose, a disaccharide (fructose-galactose) solution that promotes diarrhea, reducing the absorption of toxins.⁹ Intestinal bacteria produce most of the toxins that are absorbed by the intestines. Lactulose changes the pH in the large intestines, decreases the adsorption of ammonia and other toxins in addition to making the environment uninhabitable for the bacteria that produces the toxins.⁹

In cases where the clinical signs are not controlled with the diet and/ or lactulose, the veterinarian will normally prescribe antibiotics to reduce the number of toxin- producing bacteria present in the intestines. Although diet and medicine are valid treatments the most effective treatment that provides the best chance for a long and healthy life is surgery.⁹

Procedures for Shunt Occlusion

Silk Suture Ligation

A few investigators have reported successful occlusion of the congenital portosystemic shunt, using a silk suture. Additional studies claim that complete occlusion is not controllable using silk sutures.¹¹ Silk, a non-absorbable material, is capable of inducing an inflammatory response within a vessel wall. This is thought to be partially responsible for the occlusion effect that occurs when a silk suture is utilized for vessel ligation in response to a congenital hepatic

shunt being present. Initially activated by the degranulation of the neutrophils a chronic inflammation response (consisting of fibroblast infiltration, proliferation, and scar formation) gradually occludes the vessel primarily around the time of application of the sutures.^{11,12} In a study conducted by Youmans and Hunt, in which they tested the four primary vascular occlusion methods (Ameroid constrictors, cellophane, silk sutures, and transverse coil embolization) they noted that silk sutures were the only method that failed to completely constrict the vein that was being shrunk (attenuated) within a 6 week period.¹³

There are many factors that are thought to play a part in the rate of partial occlusion with silk sutures: the degree of inflammation produced by perivascular dissection, excessive manipulation of the vessel during surgery, postoperative vasospasms, as well as the diameter of the suture being utilized.^{11,12,13} The advantages of using silk sutures as a treatment are (a) the accessibility; (b) low cost, and (c) flexibility for application to both extrahepatic and intrahepatic congenital portosystemic shunts. The disadvantages associated of silk sutures are: (a) require measurement of intraoperative portal pressure during ligation; (b) potential secondary surgeries; and (c) lack of complete occlusion of the vessel walls.^{11,13,14}

Cellophane

Cellophane is derived from plant cellulose and it has a variety of purposes ranging from reconstructive knee surgery to the attenuation of single extrahepatic shunts. The use of cellophane bands for the attenuation of shunts in dogs began in since 1990. Sheets of sterile cellophane are cut to form a layered band of a set width (dependent on the size of the shunt), then

the band is placed around the shunt. The cellophane initiates an inflammatory reaction around the vessel that is generally associated with a chronic foreign body response. The inflammation leads to gradual occlusion of the enclosed vessel.^{15,16,17} The use of cellophane banding to occlude congenital extrahepatic portosystemic shunts is one of two methods currently accepted. Treatment using cellophane is quick, inexpensive, with a long constriction time thereby decreasing the chance of the developing portal hypertension.^{15,16,17}

In a study conducted by Frankel et al. on 32 dogs, 18 cases evaluated retrospectively and 14 dogs evaluated prospectively, the results of the cellophane banding of the vessels showed the technique resulted in complete occlusion of the attenuated vessels to a diameter 2.5 mm from an initial diameter of 6.8 ± 1.4 mm diameter within an 8 week period. Vessels of an attenuated diameter of 3.0 mm did not have 100% occlusion.¹⁶ They analyzed the results by reviewing the medical history, hematologic and serum biochemical analyses, abdominal ultrasonography, and portal angiography of the dogs. Frankel concluded that cellophane was a useful method in the occlusion of vessels smaller than a 3 mm diameter.

Transvenous Coil Embolization

Transvenous coil embolization (TCE) is a process in which a coil (spring) is placed into a shunt for gradual occlusion. A sheath is placed into the right jugular vein and a guided wire is inserted into the sheath until it reaches the shunting vessel at which a stainless steel coil is released to gradually occlude the vessel.^{14,15} The TCE method is less invasive and a more convenient therapeutic resolution technique for congenital intrahepatic portosystemic shunts but,

the down side to the process is that there are limited reports of successful treatments, especially in small breeds of dog.^{14,15} Asano et al. have been successful in the application of TCE. They tested the use of the transverse coil in a 3-month-old Miniature Dachshund. Two weeks after implanting the 8-mm diameter coil in the 6 mm diameter vessel they noticed a reduction in the serum bile acid levels and eventually complete occlusion after 4 months. The follow up study conducted 3 years post implantation showed none of the associated clinical signs (decreased function of the liver, weight loss, or hypertension). Therefore they concluded that transverse coil embolization was successful, more so in larger breeds of dogs, after testing on a range of sizes.¹⁴

Ameroid Constrictors

An ameroid is hygroscopic compressed casein that swells when immersed in fluid. The most effective treatment to date for gradual occlusion of a portosystemic shunt was done using an ameroid constrictor. A stainless steel cuff, to withhold the stresses related to the expansion (Figure 9), encases the occluder. The inner diameter of the shunt occluder was 5 mm, although they are commercially available in size ranging from 3 mm to 10 mm. The locking mechanism for the ameroid occluder (Figure 9) consists of a cylindrical peg, approximately 2 mm in diameter for insertion into the ameroid disc. The Ameroid's swelling capabilities facilitate a tight fit between the ameroid and the lock. The placement of the Ameroid occluder in a surgical operation is shown in Figure 10.

Results from original experiments using the occluder showed 14 % of the dogs died in the early postoperative period from portal hypertension.¹⁰ In comparison to the surgical ligation

technique, the ameroid constrictor produces a suitable decrease in problems such as portal hypertension, hemorrhage, or intractable seizures following recovery from anesthesia, which are associated in up to 50% of dogs post-ligation.

In an experiment conducted by Vogt, they observed early rapid expansion during the first 14 days after implantation followed by 2 months of slow expansion.¹⁰ Inou et. al also conducted a study to measure the expansion of an ameroid occluder. They recorded in the in-vitro study that the ameroid expanded rapidly to 6% stenosis during the 24-hour period after immersion into saline. The stenosis progressed slowly thereafter to 9% and 14% after 20 and 30 days respectively. For the in-situ study the coronary stenosis progressed to $57 \pm 14\%$ (mean $\pm$ SD), $78 \pm 14\%$, and $58 \pm 10\%$ on the 10th, 20th, and 30th day post ameroid implantation (Figure 11).^{18,19}

It was suggested that a more gradual approach to occlusion of the portosystemic shunt, possibly 3 to 4 months, would be beneficial to the dog. Establishing a slower pressure change is the main goal of this type of treatment. By having a slower gradual occlusion the pressure change in the shunt is slowly changed, decreasing the major problems associated with rapid vessel closure.⁶

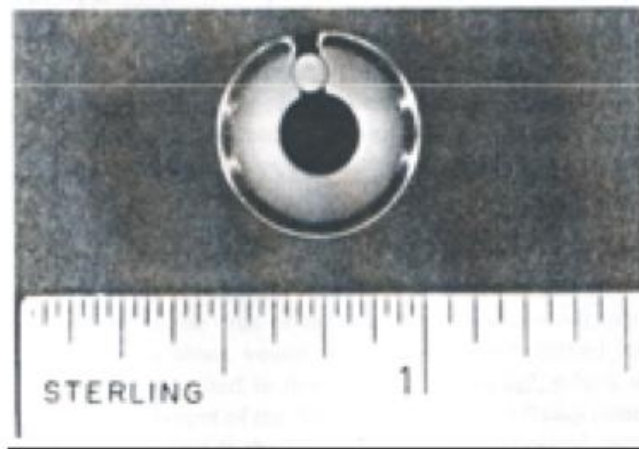


Figure 9: Ameroid Occluder shown with a ruler

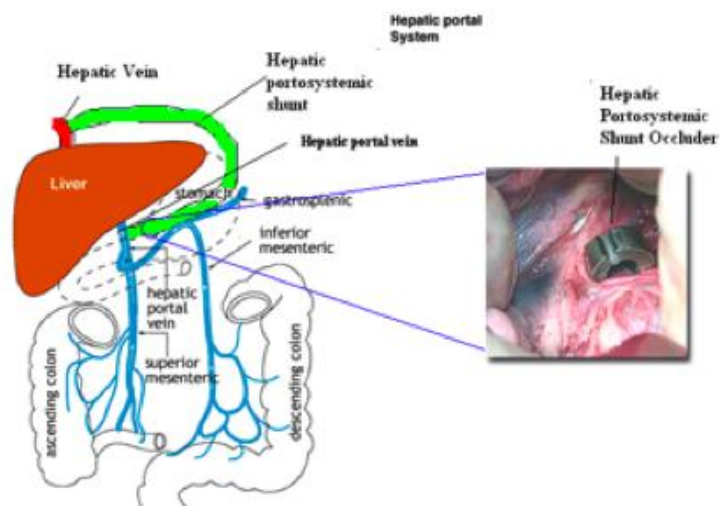


Figure 10: Hepatic Portosystemic Shunt w/ occluder placement

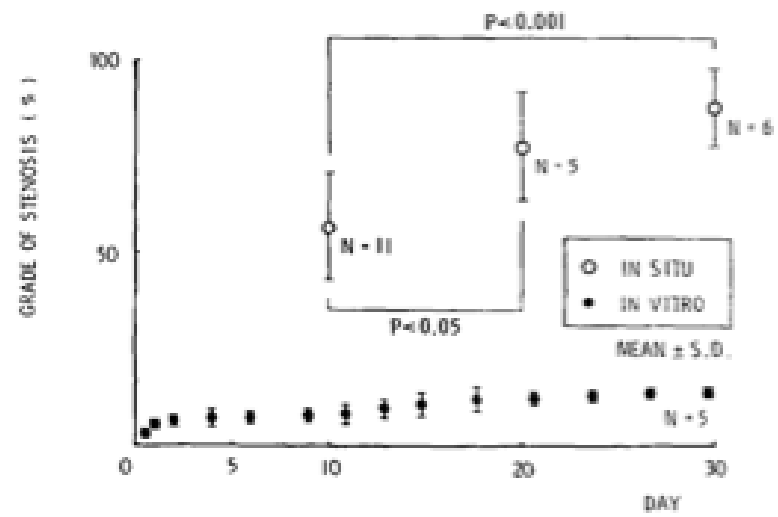


Figure 11: Ameroid Occluder Expansion in relation to Time [19]

CHAPTER 2

DESIGN MATERIALS

The aforementioned shunt occluder designs (Ameroid occluder, silk ligation, and cellophane banding techniques) all have flaws leading to outcomes that ranged from partial occlusion to rapid occlusion and associated postsurgical issues such as hypertension. The goal of this study is to design a new occluder that eliminates the problems associated with current devices/ procedures. The proposed design is based on the use of hydrogels and degradable polymers.

Hydrogels

Hydrogels are classified as crosslinked polymeric structures that swell during water adsorption. They are normally made via a free radical polymerization mechanism, where monomer units are connected into long chains through their double bonds. Crosslinking agents, monomers with two or more double bonds, provide a polymer network structure by connecting the long linear polymer chains. The crosslinked polymer chains contains either covalent bonds, physical crosslinks from entanglements, association bonds, or strong van der Waals interactions between chains.²¹ Hydrogels are classified into four categories: (i) homopolymer hydrogels, (ii) copolymer hydrogels, (iii) multipolymer hydrogels, and (iv) interpenetrating polymeric hydrogels. The classification is dependent on their method of preparation, physical structure or ionic charge.²¹ Homopolymer-hydrogels are crosslinked networks of a single type of hydrophilic monomer unit. Copolymer hydrogels are hydrogels that are produced by crosslinking two co-

monomer units, of which one must be hydrophilic to render the hydrogel swellable.

The degree of crosslinking in a polymeric network structure is a critical factor because it dictates the mechanical strength, swelling ratio, and many other properties of the polymer gel by influencing the molecular weight between crosslinks (M_c). The degree of crosslinking of a polymer is controlled by the fractionation of crosslinking agents present in the copolymerization and the double bond conversion in the reaction. Smaller amounts of crosslinking agent can lead to a less densely crosslinked hydrogel.

The swelling of hydrogels is defined as the ratio of the volume of fluid to the volume of solid inside the gel. The swelling ratio can be calculated using the following equation.

$$E_{SR}(\%) = \left(\frac{W_s - W_d}{W_d} \right) \times 100$$

Equation 1

ESR is the water absorption (wt %). W_d and W_s are the weights of the samples in the dry and swollen states respectively.⁶² A polymer swells by water flowing into the spaces between chains, which are created by the repelling of the ionic charges. Equilibrium is reached when the polymer chains are separated by enough water molecules to negate their repulsive forces on each other.²⁴ The Swelling rate is the amount of displacement the network experiences over time, Equation 2.

SR is the swelling rate, ΔX is the change in displacement, and Δt is the change over time. It is an important factor because it is proportional to the rate at which a hydrogel network

absorbs a liquid.

$$SR = \frac{\Delta X}{\Delta t} \quad \text{Equation 2}$$

Poly (Acrylic Acid)

Polyacrylic acid (PAA) is a water soluble polymer. Its large absorbent capacity and non-toxic nature makes it a common material for use in menstrual pads and diapers. PAA has the repeat unit molecular formula $C_3H_4O_2$ and chemical structure depicted in Figure 12.

The network formation of the PAA hydrogel is made by a free radical polymerization mechanism. The crosslinked structure of the ionic network is affected by the monomer concentration, pH, and ionic strength during polymerization. A hydrogel network formed from PAA has the ability to absorb many times their weight in water, or swell, and thus, is classified as a super absorbant. The ability of PAA to swell is made possible by the carboxylic acid groups on the polymer chain that associates strongly with water molecules. These types of group interaction are influenced by ionic strength and pH, therefore the swelling equilibrium of the PAA is influenced by the pH and ionic strength of the medium.^{23,24}

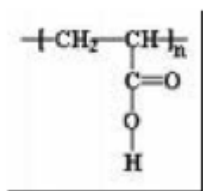


Figure 12: Poly(acrylic acid) chemical structure [22]

The molecular weight between crosslinks (M_c) dictates the mechanical strength, the swelling ratio, and the swelling rate based on the length between the crosslinks. In the shunt occluder, PAA will be used as a source of force to drive a piston to close the shunt. The force is created by the expansion of the PAA when confined in a small compartment within the occluder.

Poly (lactic acid) (PLA)

Poly (lactic acid) (PLA) is a biodegradable linear polyester derived from lactic acid. The structure of PLA is shown in Figure 13. Poly (lactic acid) is synthesized through a ring-opening polymerization reaction (Figure 14). The methyl group adds to the more amorphous characteristic as well as the increase solubility in organic solvents. The most commonly used form is Poly(lactic acid) in the L-form -P(L)LA, which releases L-lactic acid, a component common of the body, when it degrades.²²

PLA degrades by homogeneous, hydrolytic erosion. Through hydrolysis and microbial action PLA breaks down into carbon dioxide and water. PLA usually degrades in a two stage process. In the first stage of degradation, the majority of the molecular weight loss occurs with some loss of tensile strength. In the second stage, the mass and tensile strength decrease significantly, generally when the number average molecular weight (M_n) is less than or equal to

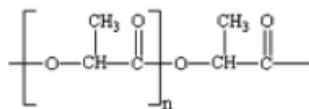


Figure 13: Poly (lactic acid) chemical structure [22]

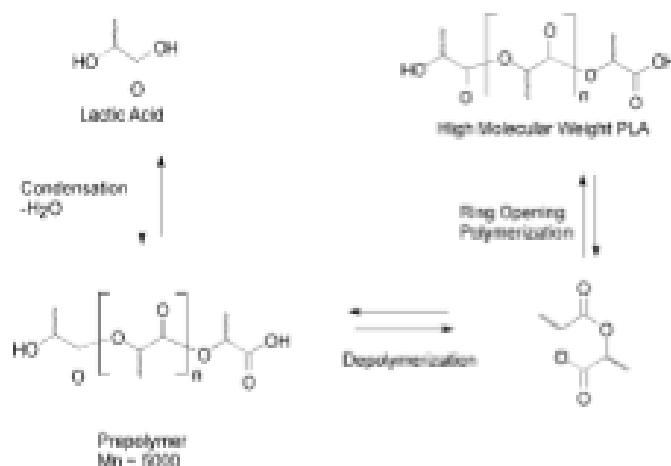


Figure 14: Reaction Scheme of Poly lactic acid [25]

15000 g/mol. P(L)LA with a molecular weight of approximately 95000 g/mol degrades in vivo by 56% over approximately a 6 month time span based on peak molecular weight (Mp) analysis conducted by Wong. For Poly (DL Lactic acid) with a molecular weight of 58000 g/mol and 87000 g/mol, there is 49% degradation in vivo in one month, also based on the Mp According to Wong.²⁵

The glass transition temperature of the PLA ranges from 51-59°C, the melting temperature ranges from 159-178°C, and the tensile modulus ranges between 1200-3000 MPa. The tensile strength, degradation rate, and the melting temperature are dependent on the weight average molecular weight and chemical structure (D or L structure).²⁵

Poly lactic acid can be processed like most thermoplastics, into fiber using conventional melt-spinning processes and film. PLA is currently used in a number of biomedical applications,

such as sutures and drug delivery devices. It is also being evaluated as a material for tissue engineering. Since it is biodegradable it can be used in the preparation of the biocompatible polymers, useful for producing loose-fill packing, compost bags, food packaging, and disposable tableware.

PLA has many potential uses in the form of fibers and non-woven textiles, such as upholstery, disposable garments, awnings, feminine hygiene products, and diapers. It is an odorless polymer and is classified as GRAS (generally recognized as safe) by the Food and Drug Administration (FDA) in the United States. PLA can be synthesized to be either rigid or flexible.

Lactic acid can be copolymerized with other monomers to control degradation. The mechanical properties of PLA, and its degradation behavior, make it an ideal polymeric candidate for implants in bone or soft tissue, and also for resorbable sutures. In summary, the mechanical, pharmaceutical, and degradation characteristics can be controlled by parameters such as chemical composition and molecular weight of the polymer.²⁶ The time frame for resorption of the PLA and its copolymers may be as little as a few weeks or as much as a few years. Major criticism of the polymer is that it releases carbon dioxide and methane during its breakdown.

Poly(glycolic acid) (PGA)

Polyglycolic acid (PGA), Figure 15, is obtained from ring-opening polymerization of glycolide. PGA is a highly crystalline, hydrophilic, linear aliphatic polyester that is biodegradable. It has a melting point of 210°C at a Mw of 50,000 and low solubility in most

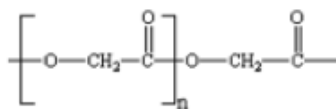


Figure 15: Poly (glycolic acid) chemical structure [25]

organic solvents. PGA has a glass transition temperature (T_g) between 35 -40°C. The degree of crystallinity ranges between 46%-52% crystalline, depending on the crystallization temperature (TC) and molecular weight (Mw).

PGA degrades mainly by bulk erosion. The erosion occurs by random hydrolysis of the ester bonds. Erosion of PGA is a bimodal process. In the first phase there is diffusion of water into the amorphous region which leads to hydrolysis. The second phase initiates after the water penetrates and starts to hydrolyze the crystalline regions.⁴³

PGA is used for the production of implantable medical devices and resorbable sutures. Surgical sutures made from PGA show mass loss mainly in the second phase when water penetrates into the amorphous region and complete degradation is achieved between 4 and 12 weeks. The rate of degradation is affected by the pH of the environment, degree of crystallinity, and curing time.²⁵ Degradation of PGA results in the production of glycolic acid which is non-toxic and is resorbed by the surrounding tissues, metabolized, and then is excreted as water and carbon dioxide.²⁵

Poly (caprolactone) (PCL)

Poly (caprolactone) (PCL), Figure 16, is a biodegradable semi-crystalline aliphatic polyester with a melting point of approximately 57°C and a glass transition temperature of -62°C.²⁵ PCL can be prepared by ring opening polymerization of ϵ -caprolactone.

PCL is capable of forming copolymers and blends with a range of other materials. It degrades by hydrolysis of the ester linkages and enzyme, which makes it an appropriate biomaterial because these are methods of degradation in a biological environment.

The degradation process occurs in two-stages. The molecular weight loss occurs in the first stage, by means of chain cleavage, and mass and strength loss occur in the second.⁴³ The degradation products of PCL are water and carbon dioxide. It is being considered as a potential for long term implants, because of a slow degradation rate.²⁵ PCL is used in drug delivery devices, sutures, adhesion barriers and is being investigated as a scaffold for tissue repair. Another application of PCL is a possible biodegradable contraceptive that can be placed under the skin.

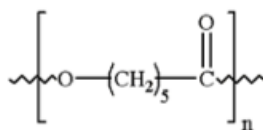


Figure 16: Poly (caprolactone) chemical structure [27]

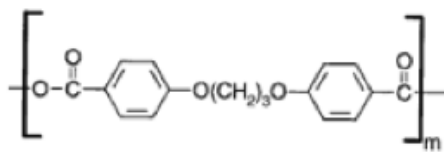


Figure 17: Poly (anhydride) chemical structure [25]

Poly(anhydrides)

Polyanhydrides are a hydrolytically unstable polymer. Polyanhydrides are characterized by the bonds that they have to the R group. They are usually either aliphatic, aromatic, or a

combination of two. Polyanhydrides have been considered to be useful biomaterials as carriers of drugs to various organs of the human body. A general structure of a polyanhydride is shown in Figure 17. Polyanhydrides are very biocompatible, can be prepared easily from low cost resources (such as dicarboxylic acid monomers with excess lactic anhydride), and can be chemically modified to meet desirable end use applications. They degrade in vivo into non-toxic di-acid counterparts that are eliminated from the body through one of the metabolic cycles. The hydrophilicity of polyanhydride copolymers makes them unique in terms of biodegradability. The anhydride bond is labile and rapidly hydrolyses in aqueous environments. However, water penetration within the core of the polymer matrix is generally not allowed due to the hydrophobicity, leading to primarily a surface erosion mechanism.²⁷

Poly (lactide-e-caprolactone)

Poly (lactide-co-caprolactone), Figure 18, is a copolymer of lactic acid (LA) and epsilon-caprolactone. This polymer is produced by a ring opening polymerization of lactic acid and

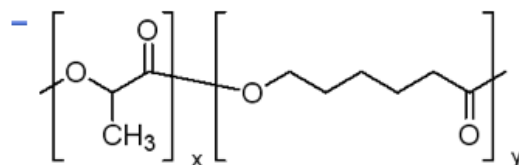


Figure 18: Poly (lactide-co-caprolactone) chemical structure [28]

epsilon caprolactone. The combination of these two polymers creates a biomaterial with more potential applications than each homopolymer. The copolymerization of the lactic acid with epsilon-co-caprolactone significantly increases its rate of degradation of the structure. Also, biocompatibility is not an issue, as both copolymers have been proven to be extremely biocompatible.

Poly (lactide-co-caprolactone) is not a widely used copolymer and does not have a known history of biomedical applications. Though each of the polymers that constitutes the copolymer are well known and historically used in biomedical applications, there is still a possibility of unexpected events such as adverse reactions, mechanical property breakdown, and other problems pertaining to concentration ratios. Using this polymer should entail a considerable amount of testing. Also, protocols will have to be created to ensure consistency once a concentration ratio is decided upon.

Copolymers are a strong choice in biomaterials because they offer flexibility and the properties can be manipulated to meet specific needs. This is not only useful in controlling rates of degradation but is also useful in managing desired properties such as benign byproducts.

Copolymerization of two polymers extends control of the mechanical properties, including tensile strength and modulus. The effect that copolymerization has on each of the individual homopolymers is especially important. In the instance of poly (lactide-co-caprolactone), copolymerization of the PLA with PCL increases the rate of degradation of the copolymer by degrading the PLA portion of the copolymer faster and eroding the structure.

The biodegradable polymer's degradation rate is very important therefore calculations related to the composition of the polymers in the copolymer blend and molecular weights associated with the polymer, which both are associated with the rate of degradation, have to be determined to reach the goal of complete occluder constriction in 3 months. Further testing will be required to obtain specific information of the relationship between the swelling of the hydrogel and the degradation of the polymers, as depicted in Figure 18. The degradation of the polymer and swelling of the hydrogel must be controlled to the point where the swelling and degradation occur at complementary rates to cause the desired gradual occlusion. Poly (lactide-co-caprolactone) seems to be the most appropriate degradable biomaterial for the new device design.

Polyoxymethylene (Acetal)

Polyoxymethylene (Acetal), Figure 19, is a copolymer that is composed of Delrin (an acetal homopolymer) and BASF Ultraform, (a cyclic ether). Polyacetal resins are implantable high performance polymers that have high chemical and mechanical resistance. They are commonly used in joint replacement components and other long term implants.³⁰ Polyacetal,

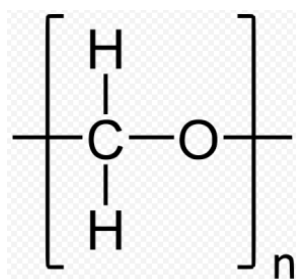


Figure 19: Polyoxymethylene chemical structure [29]

mostly in a copolymerized form, has been used in in-vivo components, such as the Bjork-Shirley tilting disc mechanical heart valve, for more than 20 years.³¹

The major advantage of having the copolymerized acetal is that it is stabilized. In homopolymer form the polyacetal tends to depolymerize and eliminate formaldehyde. The stabilization of the acetal is done by copolymerization with cyclic ether.³¹

The key properties of Acetron (polyoxycymethylene) are that it has high mechanical strength, high modulus, high natural lubricity, excellent dimensional stability, and low moisture absorption. A few of its uses are for bearings gears, electrical components, fuel system parts, pulleys, anti-friction parts, and medical applications (i.e. bone joints and heart valves). Acetron would be ideal because of its machining properties, its biocompatibility, and its certification by the FDA, the USDA, NSF, Canada AG, and etc. It has a melting temperature of 168.33°C, which is much higher than the temperature that it will be at in a biological environment (37°C). It also has a Young's Modulus of 400k psi, which is still well over the pressures necessary for occlusion.

CHAPTER 3

NOVEL DESIGN OF A PORTOSYSTEMIC SHUNT OCCLUDER

The previous studies have shown some promise in helping occlude portosystemic shunts (Figure 6), but there are still complications due to the pressure changes that occur relatively quickly after the treatments.^{11,12,14} The goal of this project is to design a shunt occluder with variable occlusion rates. The initial objective is to design a device with a three-month linear occlusion rate that would allow more time for the dog's circulatory system to adjust to the pressure changes caused by occlusion.

Materials Specifications

The material used for the shell of the device needs to be mechanically sturdy as well light in weight. The selected materials for the new occluder must maintain structural integrity throughout the life of the dog despite the (aqueous) environment in which it will be surrounded. The selected materials must not elicit unfavorable reaction therefore they must have well-established biocompatibility in order to ensure the safety of the dog. Furthermore, the material should be resistant to cell adhesion as this might impede the function of the device by blocking the occluder piston's motion or by encapsulating the device. The same material will be used for the occluder's piston. It needs to be smooth so as not to create friction to prevent the piston from movement required to occlude the shunt.

Materials

The shell of the device was a simple design. The cylindrical shell is made of two parts that come together to encase the vessel, Figure 20. This design allows for the surgeon to load the degrading polymer and swelling hydrogel into the device prior to surgery, which decreases actual surgery time. The cylinder diameter is varied based on the size of the portosystemic shunt that is to be placed in the vessel chamber. The casing is a polyoxymethylene material with the trademark name Acetron. This material is used because of its FDA approved; it has sufficient mechanical strength, and can be machined with ease.

Design

The new portosystemic shunt occluder (Figure 20) consists of three different sections: a piston (Figure 21), a polymeric chamber (Figure 22), and a vessel chamber (Figure 23). For assembly, the vessel chamber snaps together with the polymeric chamber and the piston is suspended in between the two sections. The vessel chamber, a “ U ” shaped component that can be seen in Figure 23, was designed for easy and comfortable placement of the shunt inside the device.

The shape of vessel chamber decreases the possibility of pinching the targeted shunt vessel, which could lead to blood disruption. Rapid blood flow disruption was determined to be the reason that dogs with the Ameroid Occluder are at higher risk for hypertension, which leads to the 14% mortality rate associated with the device. Check this percentage with newer papers. The polymeric chamber is where the two desired polymers (the swelling hydrogel and the

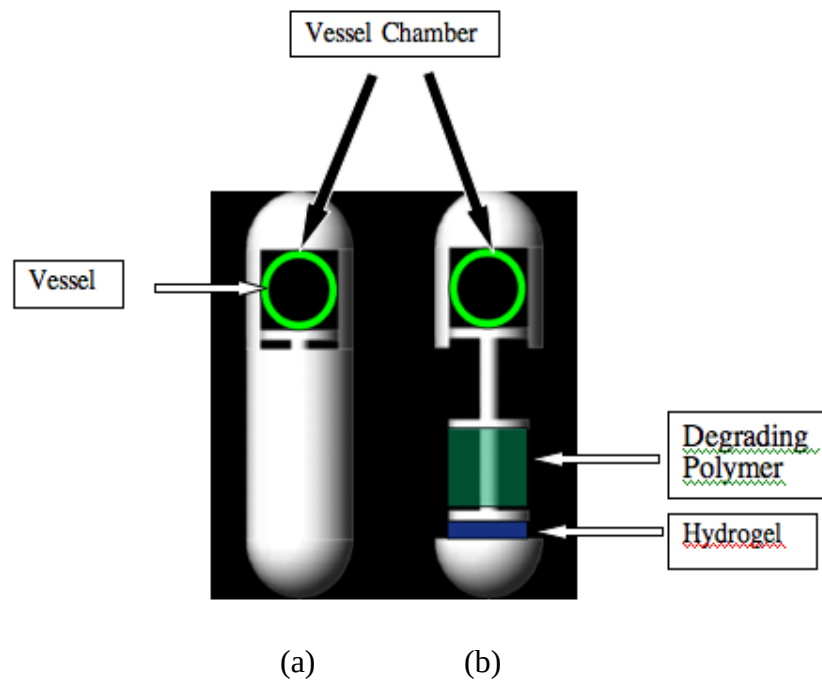


Figure 20: Hepatic Portosystemic Shunt Occluder
(a) full device (b) occluder with polymers present

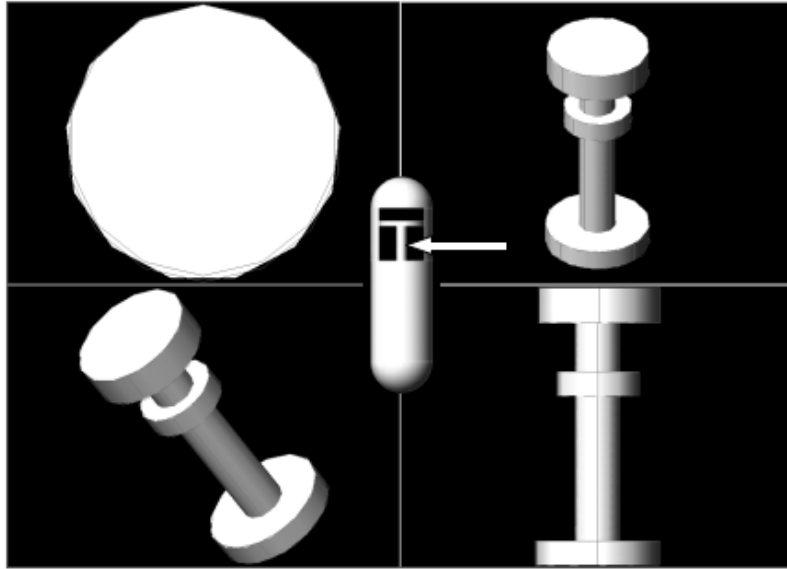


Figure 21: Occluder's Piston

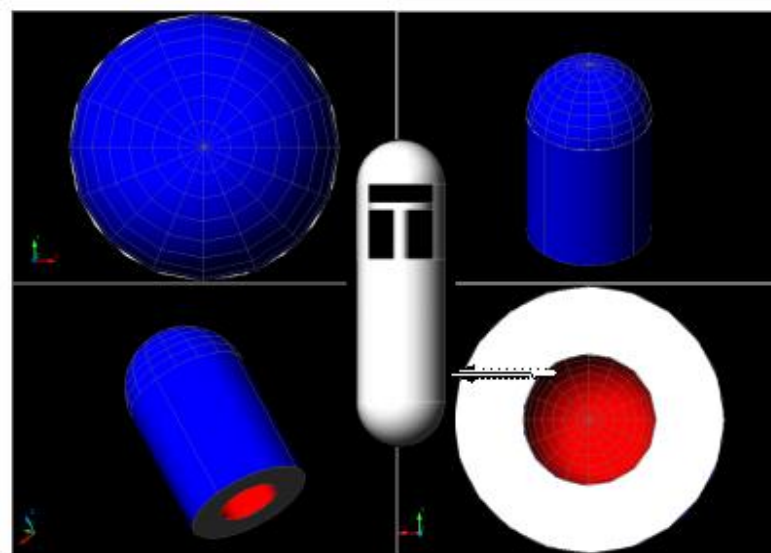


Figure 22: Polymer Chamber

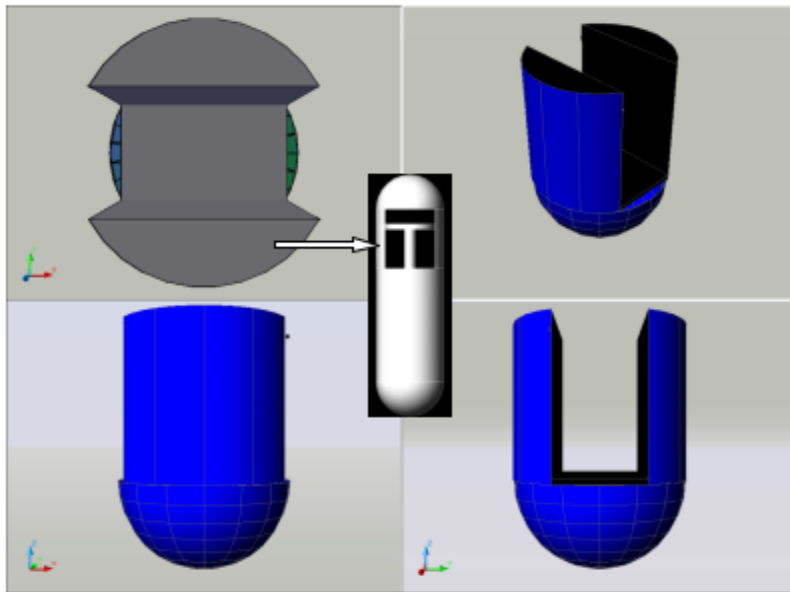


Figure 23: Vessel Chamber

degrading polymer) are loaded, Figure 22. The third part of the device, the piston, was designed to have unidirectional motion. The piston makes contact with the shunt and is directly responsible for the occlusion.

The material from which the device is being constructed is polyoxymethylene. The degrading polymer that is desired to control the piston's motion was determined by its rate of degradation, the byproduct, and loading capabilities in the design. The swelling polymer, that is being used to provide the force necessary to close the shunt, was determined based on the possible force that would be generated to move the piston.

Poly (DL lactide-co-caprolactone) (PLCL) was chosen as the degrading polymer because of its surface-eroding mode of degradation as well as its slow rate of degradation. The PLCL will be placed in the compartment adjacent to the swelling hydrogel, as can be seen in Figure 20.

The PLCL slowly erodes to impart the force from the swelling polyacrylic acid hydrogel via the piston onto the vessel. Vert et al. found that the degradation of massive P(DL)LA and P(L) LA specimens proceed more rapidly in the center than at the surface because of the concentration of carboxylic acid end groups.³² PCL, an aliphatic polyester, provides good permeation for steroids. It has a long degradation time that ranges between 3-5 years.³²

Block PLCL, originally synthesized by Feng et al., has both the advantages of the permeation rate of steroids and a degradation rate between that of the homopolymers (lactide and caprolactone), which can be controlled by adjusting the composition of the copolymer.³²

Ye et al. conducted experiments to determine the degradation rate of PLCL in a phosphate buffer solution at various temperatures and compositions. In Figure 24 the intrinsic viscosity was analyzed for various compositions at different time intervals. There is a semi-logarithmic relationship between the intrinsic viscosity $[\eta]$ of homo- or block copolymers and degradation time in phosphate buffer at the temperatures of 37°C, 45°C, and 55°C. PCL (o) 87/13, (□), 51/49 (Δ), 32/68 (p), PLA (*). The viscosity of the polymer compositions decreased as time progressed. As temperature increased the degradation rate increased. The intrinsic viscosity is proportional to the molecular weight, higher molecular weight means a higher viscosity.³²

In a study using poly (lactide-co-caprolactone) (65:35) to make nerve guides. 80% of the polymer had degraded after four months. The ability to alter and control the rate of degradation

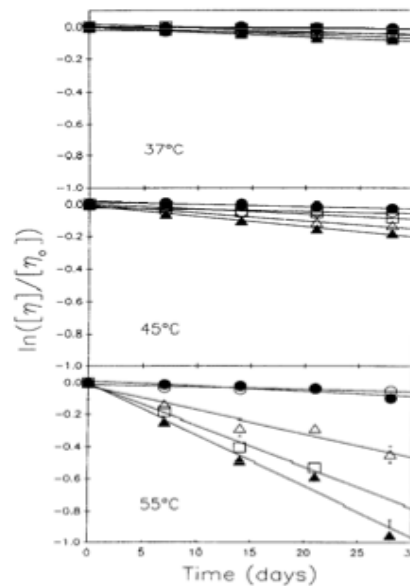


Figure 24: PLCL Intrinsic Viscosity in respect to time [32]

using different molar ratios of each copolymer is a very appealing characteristic. Tailoring the degradation rate, and correlatively the occlusion rate, has the potential to eliminate previous problems of hypertension due to spontaneous closure of the shunt.³²

Design Overview

Figure 25 is an overview of the procedures for the hepatic portosystemic shunt occluder's testing. The three main sections are the device design, the polymer components, and the complete mechanical system testing. There are two polymers that are being examined: the swelling hydrogel polymer- Poly Acrylic Acid (PAA) and the degrading polymer-Poly (lactide-co-caprolactone) (PLCL). The purpose of the PAA is to provide pressure to shift the piston that will be in contact with the portosystemic shunt. The purpose of the PLCL is to control the rate at which the piston will cause the vessel to occlude.

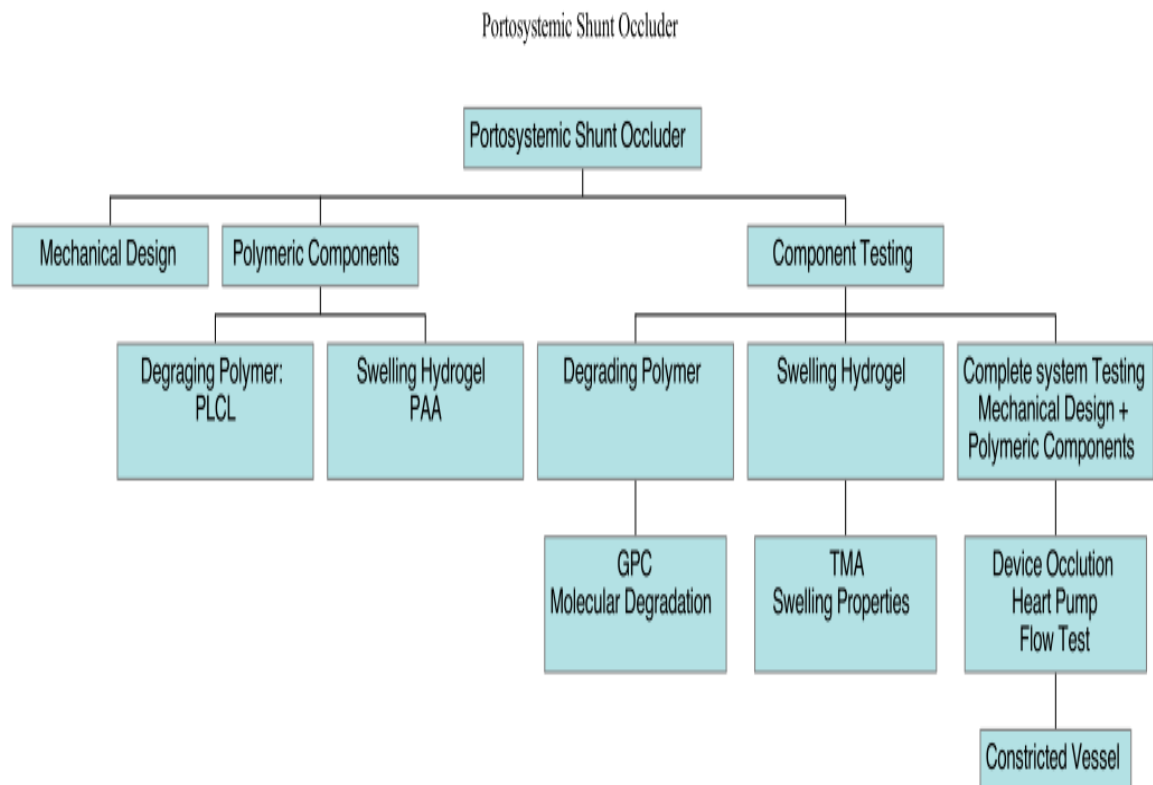


Figure 25: Portosystemic Shunt Occluder's Project Flow Chart

CHAPTER 4

EXPERIMENTAL

Materials

The PAA, the hydrogel for the experiment, was obtained from baby diapers. The degradation polymers, Poly (DL lactide-co-caprolactone) (86 mol % lactide [457647-5G] and 40 mol% lactide [457639-5G]), were purchased from Sigma Aldrich. The molecular characteristics for the PAA and the Poly (DL lactide-co-caprolactone) are presented in Tables 1.

Testing Procedure

The following are the studies that were performed to determine if the chosen polymers experimentally had the desired characteristics (swelling ratio, generated pressure/force, and degradation rates). The swelling testing was conducted using the thermal mechanical analyzer (TMA) and the degradation studies were conducted using the gel permeation chromatographer (GPC).

Degradation Studies

The purpose of the degradation studies was to determine the rate by which the Poly (lactide-co-caprolactone) would molecularly degrade in an in vitro study that simulated a biological environment (Temperature and pH).

Table 1: Molecular characteristics of Poly (Lactide-co-caprolactone) and Poly Acrylic Acid

Poly(D,L Lactide- co-caprolactone) 86:14	Poly(D, L Lactide -co-caprolactone) 40:60	Poly Acrylic Acid
Transition Temperature Tg -16°C	Transition Temperature Tg- -39°C Tm- 31°C	Transition Temperature Tg-106°C
Inherent Viscosity 0.7 -0.9 dL/g in chloroform (lit)	Inherent Viscosity 0.7 -0.9 dL/g in chloroform (lit)	Mn-130,000

Testing of Poly (lactide-co-caprolactone) (PLCL)

Approximately 25 mg of the PLCL was placed into a phosphate buffer saline (PBS) solution that simulated a biological environment. The degradation rates were determined over a 6 week period for two samples of the PLCL in PBS sample solutions: one at 37°C, the temperature of a biological specimen, and the other at 50°C to kinetically accelerate the degradation.

Preparation of Buffer Solution

Components:

- Sigma Diagnostics- Phosphate Buffered Saline [pH 7.4 at 25°C]
- Distilled water
- 1000mL container
- Rubber stopper (for container)

Procedure:

- Dissolve the contents of one PBS package into one liter of distilled water to get a pH at 25°C
- Verify the pH (with a Mettler Toledo Seven Multi pH meter with a Mettler Toledo InLab413 NTC tip)

Degradation of Polymer in PBS

Procedure

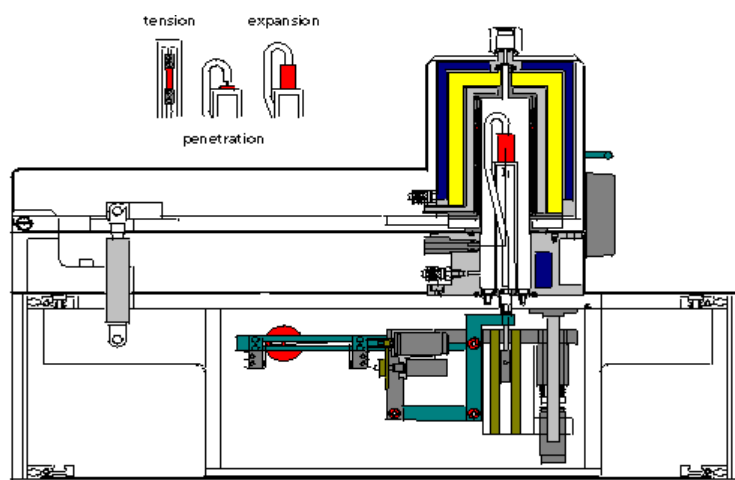
- Place 20mL of PBS into a 50mL container
- Place 20mg of PLCL into each solution
- Place container into an oven at a constant temperature of 37°C
- Place the second container into another oven at a constant temperature of 50°C
- Test the Molecular weight of the PLCL in a vile (37°C and 50°C) every two weeks, for 8 weeks.

Characterization Techniques

Thermal Mechanical Analysis (TMA)

The thermal mechanical analyzer (TMA) is an instrument that can be used to measure volumetric changes (the expansion or collapsing of a sample) in temperature controlled, atmospherically inert environment (Figure 26). TMA studies can also be used to determine characteristics such as: the glass transition temperature (T_g), the swelling ratio, swelling rate, stress temperature relationships, and pressures produced by the samples.

The sample is placed in the instrument, in a thermally conductive container such as aluminum, upon a stage that is made from a quartz glass. A rigid, thin plate is placed to partially cover the sample within the crucible. The probe, also made of quartz glass, is placed in contact with the surface of a plate. The probe and the stage are made from Quartz glass because it has a relatively low, reproducible, and accurately known coefficient of thermal expansion. It also has a low thermal conductivity that aids in isolating the sensitive transducer from the changing temperature of the furnace. Once a sample is loaded the probe can apply a static force or a



Thermo Mechanical Analyser TMA 202

Figure 26: Thermal Mechanical Analyser [33]

dynamic force in addition to the initial desired load.

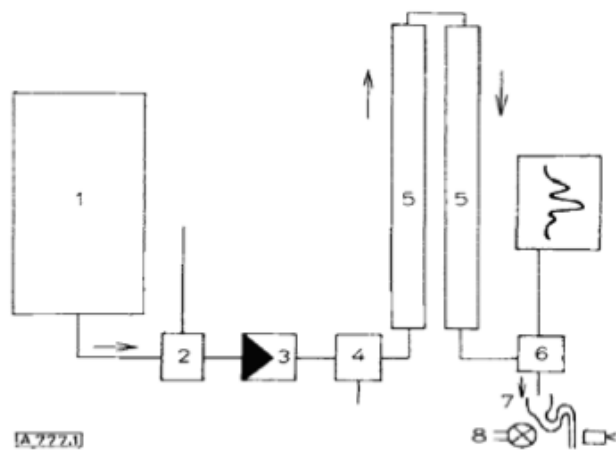
Gel Permeation Chromatography (GPC)

Gel permeation chromatography (GPC) is a experimental technique used to separate mixtures according to a component's molecular size. It consists of a mobile phase and a stationary phase. The mobile phase, which is capable of mass exchange, is a liquid that can readily dissolve the sample. The stationary phase is a porous material whose pore sizes are various sizes and separates the molecules based on their particle sizes. Porous structures are common in inorganic polymers (e.g. glass and silica gel) and in cross-linked organic polymers, usually in the swollen state.⁸

In a simple example of using GPC, shown in Figure 27, the column is packed with the swollen gel, a sample solution is poured in, and then the eluate is collected in fractions. The solvent flows from the reservoir through a sintered glass plate into a device that is used for degassing. The device is made up of an electrically heated metal block with an upright tube or a Liebig condenser heated by a thermostat-monitored liquid. When water is the eluent a peristaltic pump can be used, where as when the eluent is organic, piston pumps are more favorable. Also aqueous solutions may be injected through elastomer packing, where as organic samples have ball valves used instead.

GPC Testing Procedure

- Slowly increase the flow rate of the instrument from 0.1 to 1.0 ml/min in 0.1 increments per minute and allow calibrating for 20 mins.



Experimental arrangement in gel chromatography.
 1 = solvent reservoir; 2 = degassing device; 3 = pump; 4 = sample inlet; 5 = column; 6 = recorder; 7 = siphon; 8 = light barrier.

Figure 27: Gel Permeation Chromatography Process [34]

- Completely dissolve sample (PLCL) in THF (Do not shake sample THF dissolves the material that lines the lid). Weigh the sample so that for every 2.5mg of PLCL 1 mL of Tetrahydrofuran (THF) is added
- When completely dissolved add one (1) drop of toluene (Toluene use to monitor to filtration process).
- Clean syringe with small amount of sample possibly 0.2 mL and rotate syringe
- Place more of sample in syringe and excrete until air bubbles are removed.
- Place syringe filter on the tip of the syringe
- Place sample in GPC and lock device.
- Start Circus GPC/ Multi Detector Software Version 2
- PL Data Stream Monitor (to monitor the data collection)

Testing of the Portosystemic Shunt Occluder

Testing of all the components associated with the shunt occluder (shunt occluder shell, swelling hydrogel- PAA, and degrading polymer-PLCL) was necessary to verify complete experimental function. The experimental setup can be seen in Figure 28.

A Pulsatile Blood Pump was used to simulate the fluid flow (flow rate, systolic and diastolic pressure, volume) of a canine. A water reservoir was the fluid source (input) for the blood pump. The output fluid of the blood pump flowed to a splitter which evenly divided the pressure and flow of the fluid output from the blood pump. By dividing the fluid flow into two different paths any shift or constriction to the synthetic vessel's structure will decrease the fluid

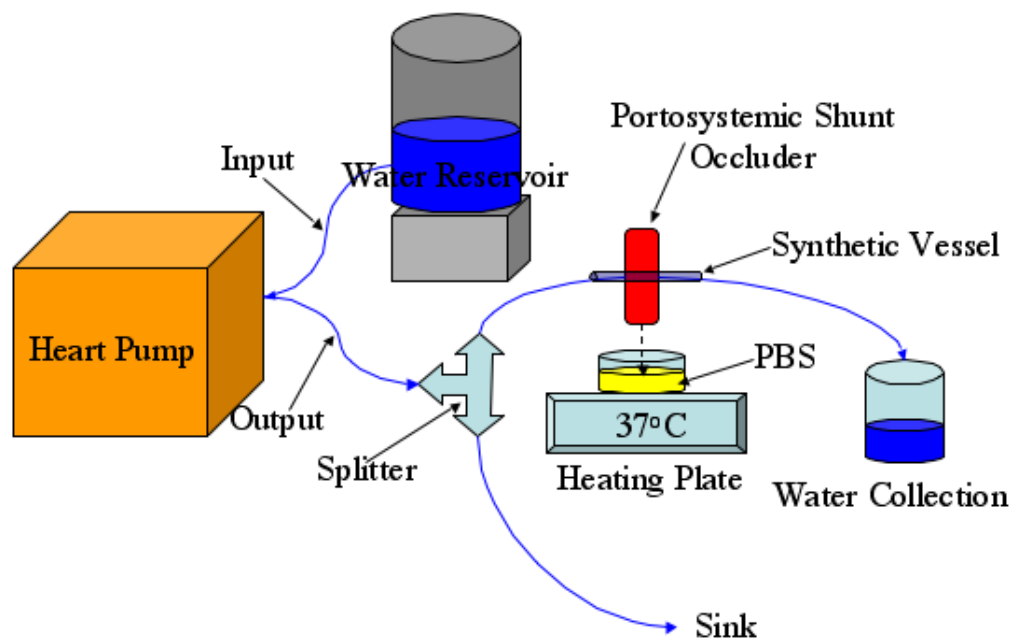


Figure 28: Complete Device Testing

five minute time period. The volume was measured in three separate trials per test date. Fluid flow rate through the synthetic vessel, and correspondingly the volume of water collected in a set flow ceased when the piston displacement was approximately 3mm. The vessel was constricted to have a closed (constricted) thickness of approximately 1mm.

Procedure

- Place PBS solution in beaker and then place the beaker on a heating plate
- Set the temperature of the heating plate to 37°C
- Place loaded (PAA and PLCL in the device) Portosystemic Shunt Occluder onto synthetic vessel.
- Connect synthetic vessel to fluid flow as presented in Figure 28
- Place shunt occluder in PBS solution (make sure polymeric chamber is completely submerged).
- Turn on the Pulsatile Pump for a 5 minute interval
- Collect water flow in separate beaker and measure with volumetric flask
- Repeat test randomly as the portosystemic shunt occluder's opening decreases (note the date and opening size)

CHAPTER 5

RESULTS

Testing was conducted on three different areas of focus to characterized the polymeric components of the shunt occluder: (1) the swelling hydrogel- poly (acrylic acid), (2) the degradation the degradation of the polymer- poly (lactide-co-caprolactone), and (3) the shunt occluder's functioning. The pressure produced, the swelling ratio, and swelling rate of the poly (acrylic acid) were determined using a Thermal Mechanical Analyzer. The molecular weight degradation rate of two different compositions of poly (lactide-co-caprolactone) (40:60 and 86:14) were determined by analyzing several samples in in-vitro conditions (pH-7.4 and temperature of 37°C) and at an elevated temperature of 50°C. The final step of in-vitro testing was to test the portosystemic shunt occluder with the polymeric materials loaded in the device, Figure 20. The portosystemic shunt occluder, loaded with the poly (lactide-co-caprolactone) and poly (acrylic acid), was tested in in-vitro conditions by analyzing the occlusions effect on fluid volume change.

PolyAcrylic Acid

Approximately 8 mg of PAA was placed into a crucible of a 0.00533m diameter and a 0.00952m height. A constant force of 13.45 N/m² was applied by the probe on the surface of the plate, and the temperature was kept constant at 37°C in a nitrogen gas environment.

Figure 29 shows the curves obtained for five different swelling tests of the PAA: the

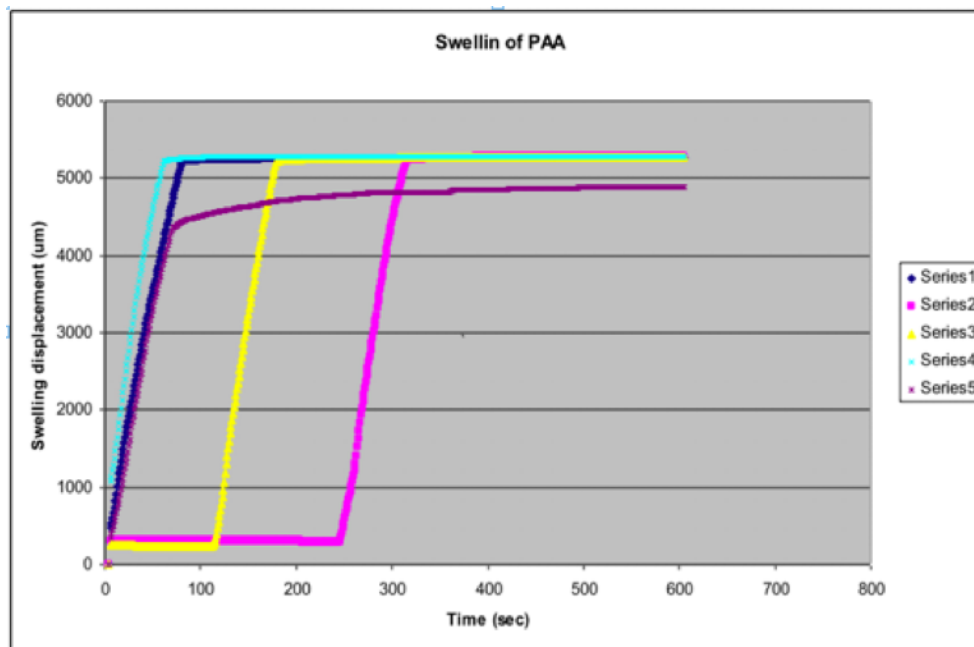


Figure 29: TMA Swelling test of PAA

information that can be obtained from this data includes the swelling rates, Table 2, and swelling ratio, Table 3. The data shows that 8 mg of PAA will produce a maximum displacement of approximately 5000 microns.

Swelling Rate of Poly(acrylic acid) Hydrogel

The swelling rates were determined for the PAA using equation 2. An average swelling rate of 0.071mm/s was obtained for the PAA hydrogel (Table 2). The PAA hydrogel loaded into the device would cause the piston to occlude a 4mm diameter vessel in 56.3 seconds, if the opposing force produced by the degrading polymer (Poly (lactide-co-caprolactone)) wasn't present.

The swelling ratio of the poly (acrylic acid) was determined by the following equation:

$$Q_H = \frac{V_f}{V_o} = \frac{\pi^2 H_f}{\pi^2 H_o} \quad \text{Equation 3}$$

Q_H is the swelling ratio of a cylindrical shaped the poly (acrylic acid) hydrogel sample. The radius of the vessel is r . H_f is the finial height of the hydrated poly (acrylic acid) in the cylindrical crucible used to hold the sample during the TMA testing. H_o is the initial height of the dehydrated PAA particles in the container. Table 3 summarizes the swelling ratios for five measurements and the average value.

Table 2: Swelling Rates of PAA Hydrogel

Trial 1 (mm/s)	Trial 2 (mm/s)	Trial 3 (mm/s)	Trial 4 (mm/s)	Trial 5 (mm/s)
0.0796	0.066	0.055	0.085	0.0696
Average	0.071		Standard Dev	0.01176

Table 3: Poly (Acrylic Acid) Swelling Ratios

Trial 1 (mm/mm)	Trial 2 (mm/mm)	Trial 3 (mm/mm)	Trial 4 (mm/mm)	Trial 5 (mm/mm)
19.66	17.43	21.12	21.3	21
Average	20.1		StDev	1.63

Degradation Studies of Poly (Lactide-co-Caprolactone) Copolymers

The degradation of two different molar compositions of poly (lactide-co-caprolactone) were analyzed using TMA. As the copolymer degraded, by both random chain scission and specific chain scission, the molecular weight of the polymer decreased. The decrease in the molecular weight was monitored by analyzing the molecular weight by GPC of several PLCL samples of different molar composition at different intervals of time (approximately every two weeks) over a 42 day and 45 day period of analysis for the PLCL (40:60) and the PLCL (86:14), respectively.

Poly (Lactide-co-Caprolactone) PLCL (40:60)

The PLCL with a molar composition of 40% Lactide and 60 % Caprolactone was placed weight associated with the degradation of PLCL (40:60). The molecular weight degradation is presented in respect to the molecular weight of the residual polymer in Tables 4 and 5.

The Poly (Lactide-co-caprolactone) (40:60) in the 37° C environment degraded at a slower rate than the same composition, in the 50°C environment. After 42 days the PLCL samples at the 37°C temperature were at approximately 50% of their initial molecular weight, where as the PLCL samples at the 50°C temperature were degraded to approximately 18% of their initial molecular weight.

Poly (Lactide-co-Caprolactone) PLCL (86:14)

The PLCL with a molar composition of 86% lactide and 14% caprolactone was in PBS solution, as previously mentioned, and kept at two different constant temperatures, 37°C and

Table 4: Degradation of Poly (Lactide-co-Caprolactone) (40:60) at 37°C

Degradation at 37°C				
Days	Mw Trial 1	Residual 1 (%)	Mw Trial 2	Residual 2 (%)
1	44299.0	100.00	44602.0	100.00
13	35714.0	80.62	35760.5	80.18
28	26931.0	60.79	28337.5	63.53
42	22368.0	50.49	22520.5	50.49

Table 5: Degradation of Poly (Lactide-co-Caprolactone) (40:60) at 50°C

Degradation at 50°C				
Days	Mw Trial 1	Residual 1 (%)	Mw Trial 2	Residual 2 (%)
1	57883.0	100.00	59106.0	100.00
16	43262.0	74.74	43705.0	73.94
30	19003.0	32.83	19070.0	32.26
42	10648.0	18.40	10737.0	18.17

50°C, Tables 6 and 7 respectively. The PLCL (86:14) was analyzed using GPC to determine the of molecular weight changes. The PLCL 86:14 composition degraded at a faster rate than the PLCL (40:60) composition. In Tables 6 and 7, the percentage of the molecular weight that remained with respect to the initial molecular weight, residual, decreased with time progression of the two separate samples.

The degradation of the PLCL (86:14) was approximately the same at 37°C and 50°C. At 37°C, over a 45 day period of time, the polymer degraded to approximately 5% of its initial molecular weight (Table 6) and at 50°C the PLCL (86:14) degraded to approximately 4% of its initial molecular weight (Table 7). There was little difference between the degradation time of the PLCL (86:14) at 37°C and 50°C.

Volumetric Fluid Flow through a Synthetic Vessel with an Occluding Portosystemic Shunt Occluder Present

The loaded (with the degrading polymer poly (lactide-co-caprolactone) and swelling hydrogel poly (acrylic acid)) portosystemic shunt occluder was positioned around a synthetic vessel (Figure 20) and placed into a phosphate buffered saline (PBS) solution and kept at a temperature of 37°C. The piston displacement, distance that the piston shifted to occlude the vessel, was measured on the different days that the testing was performed. After the piston was allowed to move a significant distance (≥ 0.5 mm) a fluid flow test was performed to determine the change in volume of the water collected, Figure 28, as a result of the piston moving position to seal off the vessel.

Table 6: Degradation of Poly (Lactide-co-Caprolactone) (86:14) at 37°C

Degradation at 37°C				
Days	Mw Trial 1	Residual 1 (%)	Mw Trial 2	Residual 2 (%)
1	97128	100.00	96513.5	100.00
16	24890	25.63	24084	24.95
31	8248	8.49	7873	8.16
45	5142	5.29	4801	4.97

Table 7: Degradation of Poly (Lactide-co-Caprolactone) (86:14) at 50°C

Degradation at 50°C				
Days	Mw Trial 1	Residual 1(%)	Mw Trial 2	Residual 2 (%)
1	97128	100.00	96513.5	100.00
16	15356	15.80	14778	15.31
31	6803	7.00	6437	6.67
45	3669	3.78	3342	3.46

There was a decrease in the fluid volume flowing through the vessel over approximately a 90-day period, at which time the flow was approximately zero (Table 8). Volume of fluid was collected over a five minute intervals of time. The average volume of the collected fluid was determined and the flow rate through the occluding vessel was determined using Equations 4 and plotted in Figure 30. Table 8 shows the corresponding flow rates in perspective to the device occlusion.

$$Q_R = \frac{Volume}{Time} \quad \text{Equation 4}$$

The distance between the piston portion of the portosystemic shunt occluder (Figure 21) and base of the vessel chamber decreased over time and correlatively the flow rate decreased. Figure 30 shows the average flow rates through the occluding vessel for several different days of testing, up to a total of 86 days.

Table 8: Fluid flow testing of Portosystemic Shunt Occluder

Time (days)	Piston Displacement (mm)	Device Opening (mm)	Fluid Volume 1	Fluid Volume 2	Fluid Volume 3	Avg. Fluid Volume	STDEV	Flow Rate (mL/min)
0	0	4	1175	1265	1110	1183.33	77.84	236.67
8	0	4	1140	1210	1160	1170.00	36.06	234.00
32	1.5	2.5	820	785	840	815.00	27.84	163.00
57	2.5	1.5	520	600	535	551.67	42.52	110.33
73	2.75	1.25	250	225	300	258.33	38.19	51.67
86	3	1	0	0	0	0	0	0

* The time interval for each flow test was 5 minutes.

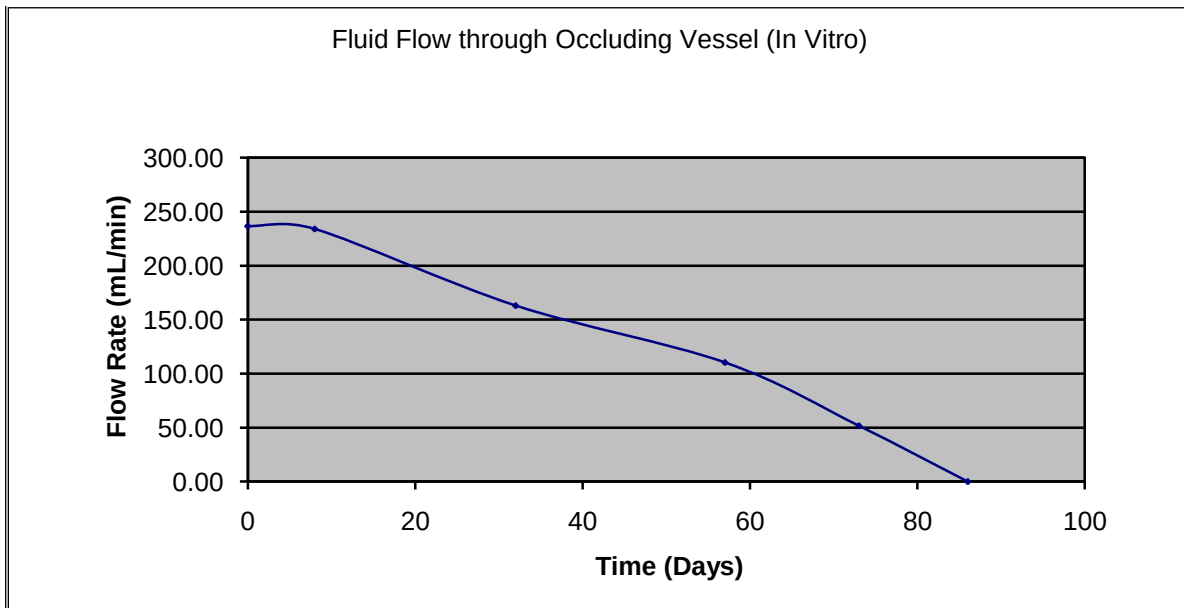


Figure 30: Fluid Flow Rate Through Occluding Vessel

CHAPTER 6

DISCUSSION

Swelling of Poly (acrylic acid) Hydrogel

The analysis of the poly (acrylic acid) – PAA swelling properties was done using the Mettler Toledo Star System TGA/SDTA851e Module Thermogravitational Analysis instrument. The pressure and swelling characteristics of the PAA were determined from the TMA analysis, Figure 29. PAA behavioral insight on how the swelling hydrogel would behave in the shunt occluder device was gained. Small opaque gel balls, that were soft and easily broken when rubbed between fingers, were formed when the PAA particles were hydrated. A set amount of unhydrated PAA was placed in a small capsule with a flat plate that acted as a contact surface for the quartz glass probe, in the TMA. PBS was then placed into the container to initiate the swelling of the PAA, Figure 26.

The swelling ratio of the hydrogel, polyacrylic acid, had been anticipated to be approximately 40 X its original size based on research conducted by Elliott et al.³⁷ However, in testing the hydrogel (PAA-H), the swelling ratio exceeded approximately 20X the original size, Table 3. The actual swelling ratio provided swelling results less than the theoretical but still more than enough to shift the piston into the desired position. The swelling rate of 0.071 mm/s was obtained from the slope of the linear graphs in Figure 28.

The PAA generated a force greater 0.033N. The PAA had to overcome an applied force of 33mN in the Thermal Mechanical Analyzer to move the quartz glass probe and obtain the data

in Figure 29. This is more than the force of 0.025 N required to occlude the vessel and significantly less than the force of 6.67 N that would cause mechanical failure of the device. The force needed to cause mechanical failure of the device was determined by physically testing the assembled device with loads (weights) applied. Mechanical failure occurred when a load of 24 oz (0.6805kg) was applied. Using equation 5 the force that was applied to the occluder device to cause mechanical failure was 6.67.

$$P = P_o + \frac{F}{A} \quad \text{Equation 5}$$

Force Generated by Poly(Acrylic Acid) Hydrogel

The amount of pressure required to overcome the pressure generated by in a canine circulatory system is approximately 0.002N/mm² (15 mm Hg).⁷ The average shunt diameter is approximately 4 mm.⁷ With this knowledge we are able to determine the force required by the swelling hydrogel (PAA) acting on the piston. The required force for the hydrogel to move the piston a distance of 4mm can be determined by Equation 6.

$$Force (N) = Area (mm^2) \times Pressure \left(\frac{N}{mm^2} \right) \quad \text{Equation 6}$$

$$F = \pi \left(\frac{4mm}{2} \right)^2 \times 0.002 \frac{N}{mm^2}$$

$$F = 25mN$$

The force required to close the shunt in dogs is approximately 25 mN. The Force required

for other diameters, within 3-10 mm, to occlude the vessel can be seen in Table 9. The swelling ratios (Q_H) obtained from TMA investigation on multiple samples, are presented in Table 3.

The average swelling ratio (Q_H) of the polymer with 8 mg of PAA was 20.10 ± 1.63 mm. The swelling ratio is determined from the height of the swollen PAA (H_f) divided by the height of the unswollen PAA (H_o), Equation 3. The amount of swelling the PAA does determine the piston displacement and rate of displacement. The swelling ratios in Table 3 are capable of moving the piston (Figure 21) to occlude the vessel, depending on if the shunt is within the 3mm to 10mm range (Table 9).

Molecular Weight Degradation

Degradation to the Poly (caprolactone) (PCL) portion of the copolymer occurs in two ways, random chain scission and specific end chain scission. PCL undergoes degradation by pure specific chain end scission by unzipping from the hydroxyl end of the polymer under isothermal pyrolytic degradation and degrades thermally by random chain scission in solution.⁶² The PLA portion of the polymer composition degrades with ester interchange processes and decarboxylation from the chain ends.⁶²

A copolymer of poly(Lactide-co-caprolactone) (PLCL) degrades at a faster rate than the homopolymer. The decomposition temperature of PCL is 402°C, where as the decomposition temperature of PCL/PLA copolymer is 350°C. One of the reasons for the faster rate of degradation of the copolymer of PCL/PLA is because the lactide acid produced, as the PLA

Table 9: Force required to Close a Canine Shunt

Force Required To Close a Canine Shunt	
Shunt Size (mm)	Required Force
3	0.014
4	0.025
6	0.057
8	0.101
10	0.157

degrades, influences the PCL⁶³ A higher molar ratio of PLA in the copolymer composition leads to more lactic acid production during the degradation of the polymer. This leads to an increase the rate of degradation.

Poly (Lactide-co-Caprolactone) PLCL (40:60)

Gel Permeation Chromatography (GPC) results were obtained using a Polymer Laboratory PL-GPC20 Integrated GPC System. Two PL-gel 5um mixed C 300 x7.5mm columns were used in the instrument with a Knaier WellChrom K-2301 Refractive Index Detector. The solvent used in the instrument was Tetrahydrofuran (THF).

The results presented in Tables 4 and 5 were obtained to determine the degradation rate of the PLCL (40:60) at 37°C (the temperature in a biological environment) and 50°C (elevated temperature to accelerate the degradation of the PLCL). Eight separate samples were prepared and placed into separate containers. Four samples were prepared simultaneously and placed in a temperature controlled bath at 37°C and 50°C for each ratio. The molecular weights, Tables 4 and 5, decreased over the 42-day period.

PLCL (40:60), at 37°C, data extrapolated to 90 days (3 months) indicated a residual of 40.7 % of the degrading polymer's initial molecular weight would remain for the targeted time of the study, as shown in Table 10 and Figure 31. The PLCL (40:60), at a temperature of 50°C, was predicted to degrade to 6.7% of its initial molecular weight after 90 days. The increase in temperature acted as a catalyst to increase the rate of degradation of the PLCL. The degradation at 37°C was compared to the rate of degradation of the 50°C. By comparing the two degradation

Table 10: Degradation /Extrapolation of PLCL (40:60)

Day	PLCL Residual Mw Percentage at 37°C (%)	PLCL Residual Mw Percentage at 50°C (%)
1	98.983	114.240
10	85.169	77.579
20	72.070	50.466
30	60.986	32.828
40	51.606	21.355
50*	43.669	13.891
60*	36.953	9.036
70*	31.269	5.878
80*	26.460	3.824
90*	22.390	2.487

*Extrapolated Data

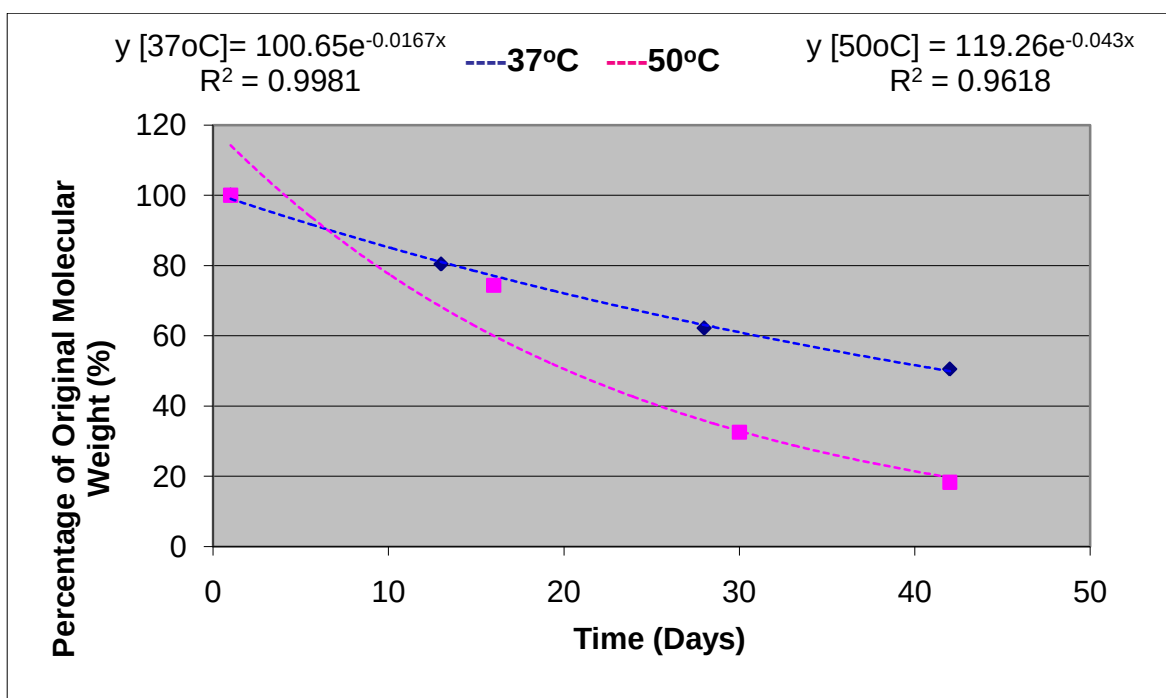


Figure 31: Degradation Behavior of PLCL (40:60) Copolymers

rates we could confirm proper degradation of the PLCL in the biological temperature of 37°C.

The effect of an elevated temperature in a biological environment, possibly due to an infection or illness, on the device's function (occluding of the shunt) could also be addressed.

Poly (Lactide-co-Caprolactone) PLCL (86:14)

The results in Tables 6 and 7 were obtained to determine the degradation of PLCL (86:14) at 37°C and 50°C. Four samples were prepared simultaneously and placed at 37°C and 50°C in a PBS Solution. The molecular weights decreased over the 45 day period. The initial molecular weight of PLCL (86:14) (Table 6 and 7) was greater than that of the PLCL (40:60) (Table 4 and 5).

The PLCL with a molar composition of 86% lactide and 14% caprolactone was also placed in PBS solution, and kept at two different constant temperatures, 37°C and 50°C. The PLCL (86:14) was analyzed using GPC to determine the of molecular weight degradation. The PLCL 86:14 composition degraded at a faster rate than PLCL 40:60 composition. In Tables 11, the percentage of the molecular weight that remained with respect to the initial molecular weight, residual, decreased with time progression of the two separate samples.

The degradation of the PLCL (86:14) was approximately the same at 37°C and 50°C, Figure 32. At 37°C, over a 45 day period of time, the polymer degraded to approximately 5% of its initial molecular weight (Table 6) and at 50°C the PLCL (86:14) degraded to approximately 4% of its initial molecular weight (Table 7). There was little difference between the degradation time of the PLCL (86:14) at 37°C and 50°C.

Table 11: Degradation/Extrapolation of PLCL (86:14)

Day	PLCL Residual Mw Percentage at 37°C (%)	PLCL Residual Mw Percentage at 50°C (%)
1	82.984	71.962
10	44.837	37.138
20	22.624	17.807
30	11.416	8.539
40	5.760	4.094
50*	2.906	1.963
60*	1.466	0.941
70*	0.740	0.451
80*	0.373	0.216
90*	0.188	0.103

* Extrapolated data

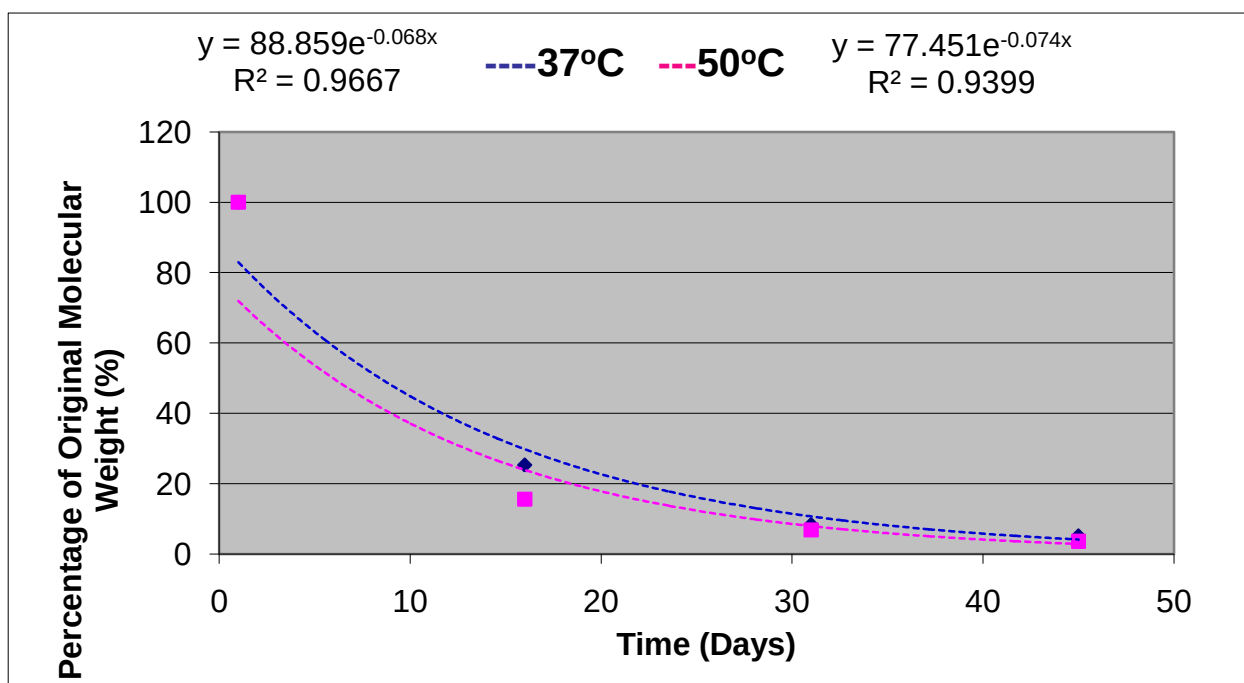


Figure 32: Degradation Behavior of PLCL (86:14)

Comparison of Degradation Behavior of Copolymers

PLCL (40:60 and 84:16)

Sivalingam and Madras conducted a study to determine the thermal stability of a copolymer composed of PCL and PLA. From their results they noted that the PCL degrades from both random chain scission and specific chain end scission. The specific chain end scission leads to the formation of ϵ -caprolactone with volatiles such as CO_2 . Sivalingam and Madras also came to the conclusion that at lower temperatures random chain scission dominates the degradation process and as the temperature increases the specific chain end scission (from the hydroxyl chain end portion of the chain) takes over and begins to dominate. This lead to the conclusion that there is a change in degradation mechanisms in PCL as the temperature of the environment, that it is placed in, increases.

For the current experiment, degradation studies of the PLCL were conducted at both 37°C and 50°C for both compositions of the polymer (40:60 and 84:16). The theoretical prediction was that the 40% lactide 60% caprolactone would degrade at a slower rate in both a biological temperature (37°C) and in an elevated temperature (50°C). Figure 33 is a plot of the molecular weight degradation of the two compositions at a temperature of 37°C. Figure 34 is a comparison of the two different PLCL compositions (40:60 and 84:16) at 50°C. The theoretical prediction for the PLCL (40:60) was correct, as can be seen by a plot of the molecular weight at the different time spans. Though the PLCL (84:16) had a higher initial molecular weight than the PLCL (40:60), Tables 4 and 6, the molecular weight of the PLCL (84:16) was less than that of the PLCL (40:60) after approximately 13 days.

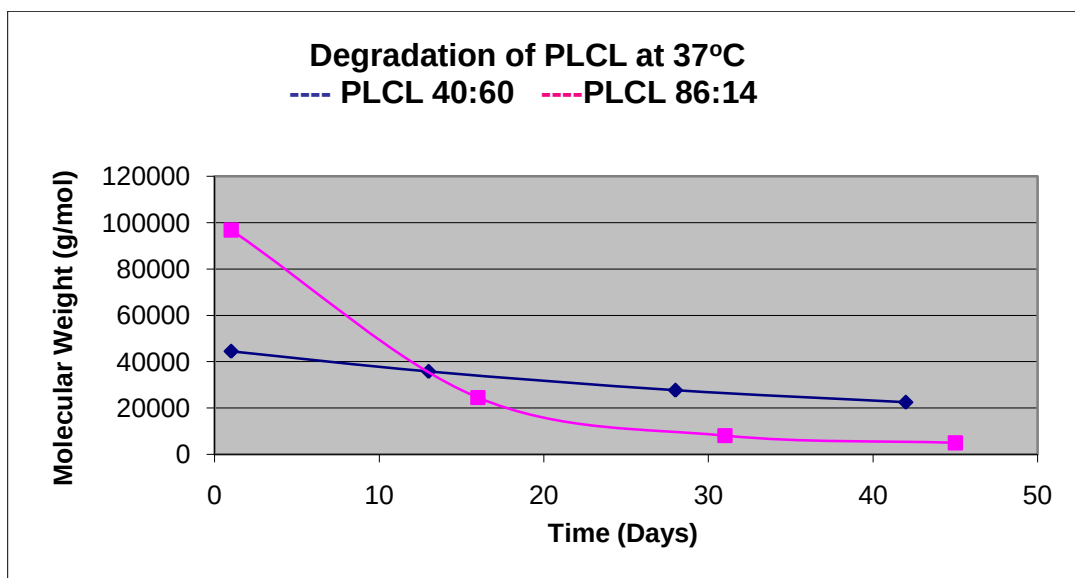


Figure 33: Comparison of PLCL (40:60) and PLCL (86:14) at 37°C

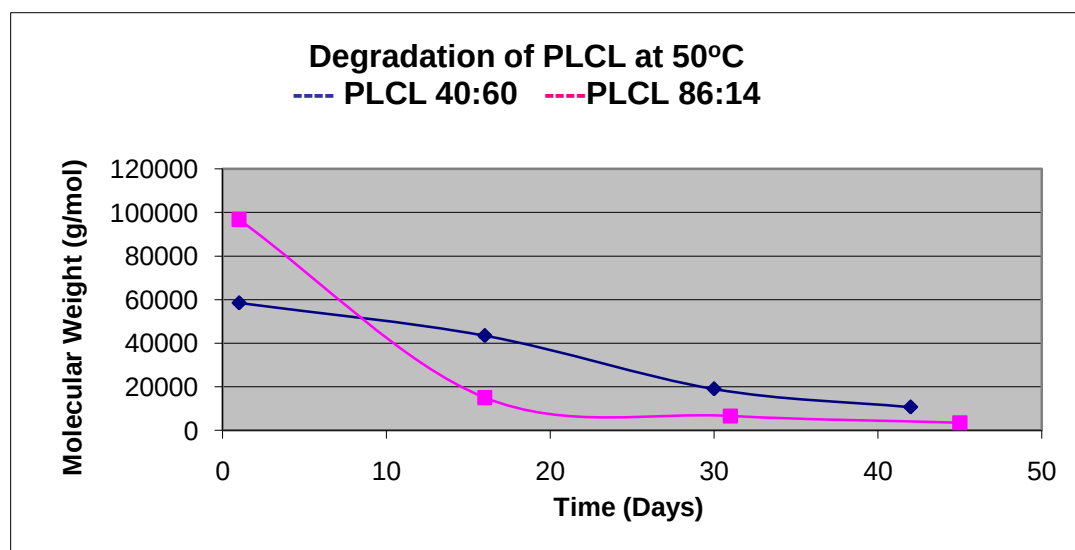


Figure 34: Comparison of PLCL (40:60) and PLCL (86:14) at 50°C

The molecular degradation rate of the PLCL (40:60) composition was slower than the molecular degradation rate of the PLCL (86:14) composition at an elevated temperature of 50°C. The PLCL (86:14) degraded to 15.31% of its initial molecular weight after 16 days; where as the PLCL (40:60) had only degraded to 73.94% of its initial molecular weight after the same amount of time. The 40:60 has a slower and more desirable rate of degradation than the PLCL (86:14).

Effect of Temperature on the Molecular Degradation

Poly (Lactide-co-Caprolactone) (40:60)

As the environment's temperature increases the rate of degradation also increase, Table 12. The effect of temperature on the molecular degradation rate of the poly (lactide-co-caprolactone) (40:60) polymer can be seen in Figure 35. At 37°C, after 10 days of degradation, the PLCL (40:60) had a degradation rate of 1.38 g/mol/day; where as at the same time (10 days) the PLCL (86:14) had a degradation rate of 3.67g/mol/day. The equation acquired, from analyzing the data plotted in Figure 35, can be used to predict the molecular degradation rate of the PLCL (40:60) at various temperatures.

It can also be seen that the degradation rate decreased as the composition of the polymer degraded, Table 12. When the PLCL (40:60) was at 85.17% and 77% of its initial molecular weights their degradation rates were 1.38 g/mol/day and 3.66g/mol/day, but at lower molecular weights, 51.61% for the 37°C environment and 21.35% for the 50°C environment, the degradation rates had decreased to 0.938 g/mol/day and 1.147 g/mol/day respectively.

Table 12: Degradation Rates of Poly (Lactide-co-Caprolactone) (40:60) at 37°C and 50°C

Days	Degradation Rate of PLCL 37°C (g/mol/ day)	Degradation Rate of PLCL at 50°C (g/mol/ day)
10	1.381	3.666
20	1.310	2.711
30	1.108	1.764
40	0.938	1.147
50	0.794	0.746
60	0.672	0.486
70	0.568	0.316
80	0.481	0.205
90	0.407	0.134
Avg	0.851	1.241

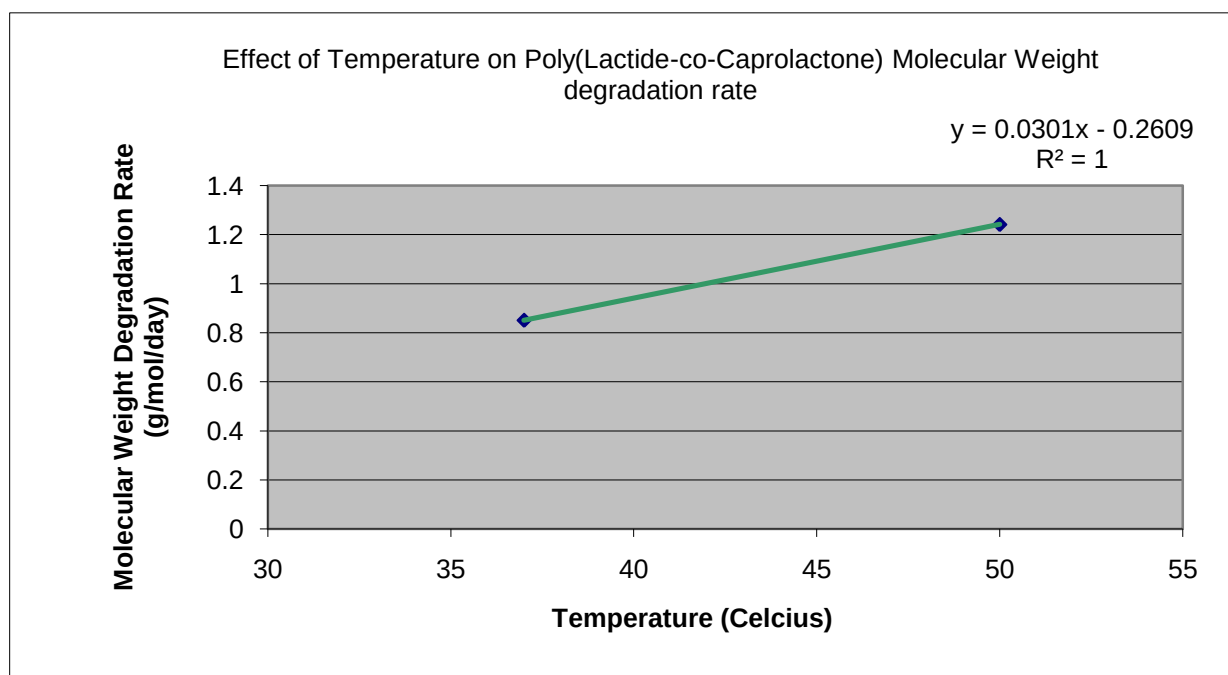


Figure 35: Molecular Degradation Rate in respect to temperature for PLCL (40:60)

The degradation rate of the PLCL (40:60) had decreased by 32.1% within 40 days in the 37°C environment and by 68.8% within 40 days for the PLCL (40:60) in the 50°C environment.

Poly(Lactide-co-Caprolactone) (86:14)

The effect of temperature on the molecular degradation rate of the poly (lactide-co-caprolactone) (86:14) polymer can be seen in Table 13 and Figure 36. Unlike PLCL (40:60) as the temperature of PLCL (86:14) increased from 37°C to 50°C the degradation rate decreased slightly, Figure 36. The effect of temperature on the molecular weight degradation rate for the PLCL (86:14) was different from what was expected. The rate of degradation was expected to increase as the temperature of the media increased. Unexpectedly, as the temperature of the media increased the rate of degradation slightly decreased, Figure 36. The difference in the molecular weight degradation rates were not significant enough (Table 13), therefore the effect of temperatures between 37°C and 50°C on the PLCL (86:14) composition can be neglected.

Testing of the loaded Portosystemic Shunt Occluder

A synthetic vessel was placed into the portosystemic shunt occluder. The shunt occluder was placed into phosphate buffer solution (PBS) that was kept at a temperature of 37°C. A schematic of the experimental setup can be seen in Figure 28. As time progressed, the initial shunt occluder diameter of 4mm decreased until the synthetic vessel was completely constricted. Complete constriction was determined by a fluid flow of zero through the vessel, Table 8.

Table 13: Degradation Rates of Poly (Lactide-co-Caprolactone) (86:14) at 37°C and 50°C

Days	Degradation Rate of PLCL 37°C (g/mol/ day)	Degradation Rate of PLCL at 50°C (g/mol/ day)
10	3.814	3.482
20	2.221	1.933
30	1.120	0.926
40	0.565	0.444
50	0.285	0.213
60	0.144	0.102
70	0.072	0.049
80	0.036	0.023
90	0.018	0.011
Avg	0.919	0.798

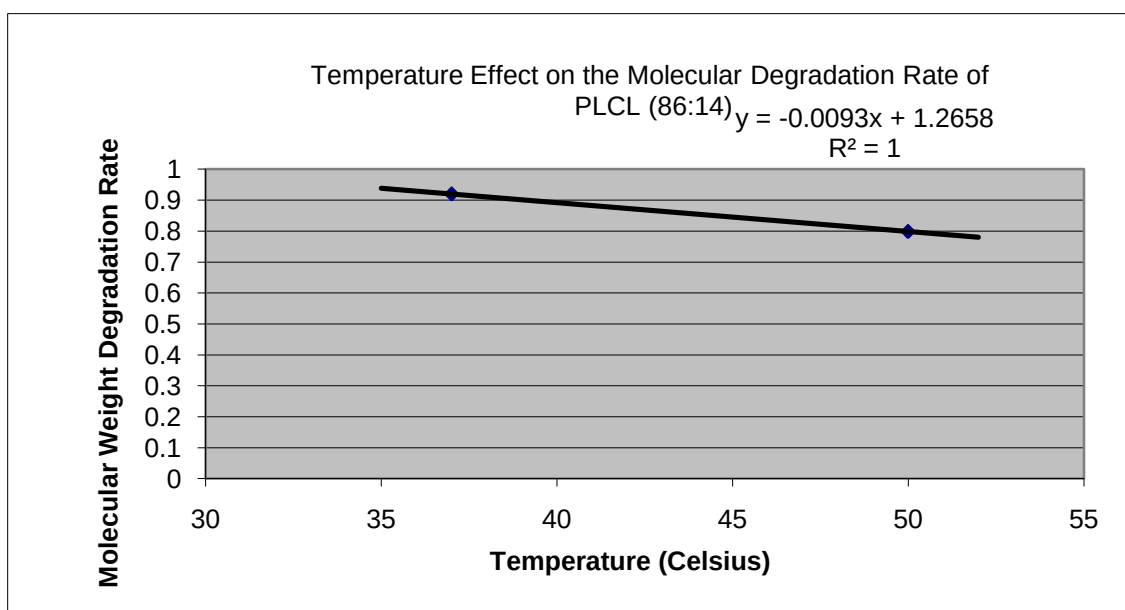


Figure 36: Molecular Degradation Rate in respect to temperature for PLCL (86:14)

CHAPTER 7

CONCLUSIONS

The goal of this project was to develop an implantable device that would slowly occlude a congenital single portosystemic shunt, which is a vessel in canines that is a genetic birth defect that causes blood to bypass the liver, over a 3-4 month (90-120 days) time. The reason for designing this device was because the current device that is used, the Ameroid Occluder, closes the vessel too rapidly and as a result it causes the system in which it is implanted to develop hypertension.. Hypertension results in development of multiple acquired shunts, which permit continued diversion of portal blood around the liver instead of through it.

The new portosystemic device, that this project developed, was designed to be small (varies based on the shunt size it is designed for), biocompatible (materials compatible with biological system), light in weight (~1.15 g for a 4x4mm opening), simple (3 components, one of which that moves), and most importantly able to occlude the portosystemic shunt over a 3-4 month (90-120 day) time span. The different components of the device (the swelling hydrogel-PAA, the degrading polymer-PLCL, and the mechanical parts) were tested in separate stages.

The PAA had a swelling ratio of 20.10 ± 1.63 and a swelling rate of 0.071mm/s^2 . It produces approximately 25mN of Force, which is more than the 8mN needed to occlude a vessel, but less than the required force of 6.67N to cause mechanical failure of the device. The

degradation polymer was predicted to molecularly degrade significantly so that the mechanical properties of the polymer do not counter the force produced by the PAA after a 90 day time span in a 37°C environment. When the complete device was tested, Figure 30, the device occluded the synthetic vessel in 86 days. This is slightly shorter than the desired time range of 90 –120 days but the molar composition of the degradation polymer can be adjusted to change the time of occlusion.

Proposed Future Work

Additional testing needs to be conducted in an in-vivo environment before the device can be marketable. The in vivo testing will be potentially conducted by Dr. Tobias with the University of TN, School of Veterinary Medicine. The testing conducted thus far has been in vitro and though it provides some incite to how the polymers and the device as a whole will behave in the biological environment, it does not account for enzymatic degradation, foreign body response, or the fluxing dynamic motions of a biological specimen. Animal testing needs to be conducted in order to determine the system faults.

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Vita

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