Canine Tracheal Stent: Biocompatibility

A Major Qualifying Project Report submitted to the faculty of
WORCESTER POLYTECHNIC INSTITUTE
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Abstract

Canine tracheal collapse is a condition resulting in the weakening of tracheal cartilage rings. As the cartilage weakens, the trachea collapses, causing difficulty breathing, discomfort, and other medical issues. Self-expanding nitinol stents are utilized to restore and sustain the trachea’s internal area. These stents fail to effectively resist migration, resulting in the stent dislocating and irritating the surrounding tracheal tissue. Following validation testing of three polymers, chitosan, polycaprolactone (PCL), and poly-L-lactic acid (PLLA), it was determined that the chitosan polymer coating best fulfilled the design objectives, due to its ability to produce dip coating, low mechanical integrity impact, low cytotoxicity, mucoadhesive properties, and low production cost.
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1.0 Introduction

Yorkshire Terriers are the ninth most popular registered dog breed in the United States according to the American Kennel Club’s 2016 rankings [1]. A disease known as tracheal collapse is most commonly found in small breed dogs, with the highest prevalence in Yorkshire Terriers [2], [3]. A healthy trachea is a tube that runs from the larynx to the bronchi composed primarily of C-shaped cartilage rings and connective tissue [4], [5]. Functionally the trachea allows inhaled air to pass through, while filtering, warming, and adding moisture to it to make it favorable for the lungs [6]. Tracheal collapse weakens the structure of tracheal cartilage, leading to the inward collapse of the cartilage rings [2], [3]. Depending on the severity of the collapse, the effects can range from irritation and “goose-honking” coughs to fainting, difficulty breathing, or death [3], [5]. Tracheal collapse progression in Yorkshire Terriers is exacerbated by genetic and environmental factors, and requires treatment to minimize the discomfort and suffering of the dog [2], [3]. The condition is largely manageable in the early stages without the need for surgery.

Tracheal collapse is always managed non-invasively prior to surgical options. Medical management options include weight loss regimens, cough suppressants, and sedatives to limit the dog’s excitement [3]. While implementing exercise and dietary regulations requires additional care and supervision, medical options can be costly dependent on the drug used. Management may not permanently offset the propagation of severe trachea collapse, requiring further corrective action if it does progress to a severe case. When management fails to be effective, surgical intervention is utilized. Two implantable device types exist regarding tracheal collapse, extraluminal prosthesis and intraluminal stenting [2], [5]. Extraluminal prosthesis utilizes ring-like strips of plastic applied to the surface of the trachea externally pull it back into shape. Intraluminal stents are mesh, tube shaped devices used to expand the opening of a trachea, forcing the airway back into its original shape. The gold standard for this type of treatment is the Vet Stent Trachea designed by Infiniti medical. The Vet Stent utilizes nitinol as a material to self-expand from the catheter sheath to full size. Approximately one quarter of implanted tracheal stents will fracture, while one third will develop unwanted granulation tissue in the stent due to the inflammatory response of the surrounding tissue [2]. A need exists at Tufts Veterinary’s Foster Hospital for Small Animals for a device that is capable of outlasting current options and improving the overall quality of life of the recipient.
The client, Dr. Elizabeth Rozanski at the Foster Hospital for Small Animals and Tufts Cummings School of Veterinary Medicine, seeks an improved treatment method over existing stents to improve the quality and longevity of treating tracheal collapse in small breed dogs, specifically Yorkshire terriers. The prevalence of tracheal collapse in a widely popular dog breed results in the need for effective, affordable treatment option for the disease. The goal of this project lies in developing and testing modifications for use with existing canine tracheal stents. A successful modification will decrease granulation tissue growth around the stent, while keeping the stent in place following implantation. Some additional design requirements include minimizing airway irritation, preventing cell death due to toxic materials, encouraging the growth of beneficial epithelial cells to coat the stent, and extending the lifespan of the device to match that of the recipient, approximately seven or eight years. The modification must also interface with currently marketed stents and be produced at a competitive price; the Infiniti Vet Stent utilized by Tufts veterinarians costs approximately $1,100, however the hospital will pay a maximum of approximately $1,500 for a stent capable of lasting longer and outperforming the current options [3]. With this in mind, the modification may cost up to a maximum of $400 to manufacture.

To accomplish this goal, several components must be applied to this project. Modification options must be considered and tested for both mechanical integrity and biocompatibility. The stent modification must be capable of withstanding the forces that the stent itself undergoes. They must also be tested to determine if the coatings are able to enhance the cellular adhesion of the stent to reduce the risk of dislodging. The modification is to be tested in a cellular environment to ensure that they were not cytotoxic and would not cause tissue abrasion or an inflammatory response. If successful, the modification will allow for cell adhesion, prevent cell death, and prove viable as an option for Tufts Veterinary Hospital to improve existing stents on the market. The overall success of this project may allow improvements in not only canine veterinary medicine, but also may be applied to other veterinary fields, such as feline and equine medicine [5]. Such a modification can additionally be applied to any stent that is developed similarly to a canine tracheal stent, allowing for universal application as needed by clinics.
2.0 Literature Review

2.1 The Trachea and Tracheal Collapse

2.1.1 Tracheal Anatomy and Physiology

The trachea is a tube-shaped structure that extends from the larynx to the bronchi located anteriorly to the esophagus, along the neck of the dog. It is composed of anywhere between 35 and 45 C-shaped hyaline cartilage rings connected by annular ligaments [4], [5]. The width of the cartilage rings is roughly 4mm, and the annular ligaments are roughly 1mm in width [4]. The diameter of the structure falls in the range of 9mm to 17mm, dependent on the size of the dog [3]. Mechanically, the cartilage rings serve as a solid, non-collapsible structure, while the collapsible ligaments that connect the cartilages allow the trachea to flex and bend [4]. The trachea splits at the end closest to the bronchi to allow it to connect to both lungs [5]. The dorsal (upper) component of this structure, unlike the c-shaped cartilages, is composed of mucosa, connective tissue, and tracheal muscle, and serves as the point where the C-shaped cartilages connect and form a complete tubular structure[5]. The lining of the trachea is composed of “pseudostratified, ciliated epithelial mucosa” [7].

The trachea is known as the “windpipe” and serves as a crucial component of the respiratory tract. The primary function of the trachea is to conduct air from the pharynx and larynx to the bronchi, which allows air to reach the lungs. The cilia that line the surface of the trachea, along with mucus that is produced by mucous membranes, are utilized to trap and remove irritants from the windpipe [7]. Additionally, the trachea is involved in warming, cleaning, and humidifying the inspired air. These actions keep the lungs from desiccating, and help ensure they function under optimal conditions at all times [7], [8]. A healthy trachea resists changes in applied pressure during inhalation and exhalation, allowing it to maintain its shape and prevent the loss of tracheal lumen, the opening of the trachea itself [7].

2.1.2 Tracheal Collapse

Tracheal collapse is a disease that results in a loss of tracheal lumen due to the mechanical failure of the tracheal structure. There are two ways in which this disease may propagate based on the axis upon which the collapse occurs. Lateral collapse, where the side walls of the C-cartilages collapse medially, is the less common of the two, and is often developed following surgical
intervention for dorsoventral collapse [9]. Dorsoventral collapse, the most common classification of tracheal collapse, occurs when the dorsal membrane loosens and collapses into the tracheal space [9].

The exact cause of the disease is unknown, but it is suggested to be influenced by genetics due to the tendency for toy and miniature breed dogs, particularly Yorkshire Terriers, to experience it [2], [9]. Additionally, the disease may be exacerbated by environmental and lifestyle factors including high air temperatures, living with smokers, forces applied by collars, obesity, and excitement [2], [5]. The cartilage rings of the trachea are found to reduce in rigidity and deform due to reduced contents of glycosaminoglycan, glycoprotein, chondroitin sulfate, and water in the hyaline cartilage [2]. This reduced rigidity furthers the propagation of the collapse, and further inhibits respiratory ability. Tracheal collapse is graded on a four-level scale based on lumen diameter reduction: Grade I for up to 25%, Grade II for up to 50%, Grade III for up to 75%, and Grade IV for up to 100% loss [5]. The level of collapse is best observed by utilizing endoscopy, as it allows for a dynamic view of both inspiratory and expiratory action [5]. This allows for the full breathing cycle to be examined, as the trachea may change in shape during respiration due to weakened tracheal structures, presenting less collapse during certain phases than others. Tracheal collapse causes respiratory issues including coughing in normal cases, and difficulty breathing and sudden collapsing in severe cases [2], [7]. Additionally, the increased airway irritation that occurs with the disease leads to mucous membrane inflammation, which in turn inhibits mucous production, leading to further issues with irritants [9].

2.2 Treatment Options

2.2.1 Management

Medical management is utilized prior to any surgical methods to attempt to delay the progression of the collapse. Non-medical management options include utilizing harnesses instead of collars, limiting exposure to irritants, and implementing dietary weight-loss regimens to reduce the pressure and irritation applied to the trachea [5]. The types of medications utilized include antitussives, bronchodilators, and steroids [2], [5]. Antitussives are utilized to reduce the dog’s cough and provide relief from the irritation coughing causes the trachea [2]. Bronchodilators reduce the trachea’s resistance to airflow by reducing intrathoracic pressure, improve the cilia’s ability to clear particles and mucous from the airway, and allow for the diameter of the trachea to
Steroid therapy has been shown to reduce airway irritation, and in a recent study of stanozolol, shown to increase the levels of chondroitin sulfate in the tracheal cartilage [2]. While these drugs relieve the symptoms of tracheal collapse and work to successfully manage early and less severe cases, more severe late-stage cases require further surgical intervention [2], [5].

2.2.2 Tracheal Rings

Tracheal rings, also known as extraluminal prostheses, are C-shaped rings that are typically made of plastic and attached to the external surface of the trachea using stitches [2], [5]. These rings are able to increase the tracheal lumen and prevent pressure-based collapse, offering a longer-term solution to collapse [5]. While positive outcomes are reported in anywhere from 75 to 89% of dogs after the placement of extraluminal prostheses, including a median survival time of 4 years and 6 months, this type of treatment entails additional risks [2]. The surgery is complicated, and requires the surgeon to cut open the surrounding structures to reach the tracheal surface, which can lead to additional non-tracheal complications [2], [5]. Laryngeal paralysis, a major complication, can occur in 11 to 30% of extraluminal prostheses recipients, which may require additional surgery to correct [2], [5]. As a result of the difficulty and risks of this surgery, intraluminal tracheal stents are the most commonly used device [2], [5].

2.2.3 Stents

When surgical intervention is required to treat a Yorkshire Terrier’s tracheal collapse, the most commonly utilized method is intraluminal stent implantation. The stent is implanted by sliding it down the dog’s throat typically using either a sheath catheter or a balloon catheter. The balloon catheter operates by sliding the stent into place and then inflating the balloon to expand the stent to full size. Once the stent is completely in place, the balloon is deflated, the catheter is removed, and the surgery is complete [10]. The sheath catheter is utilized in the delivery of shape memory alloy stents, most commonly Nickel-Titanium (nitinol). These materials can be freely bent and stretch while they are at low temperatures but return to a predetermined form when a stimulus is applied. In the case of stents, the stimulus is reaching a temperature known as the transformation temperature. Using the sheath catheter method, the stent is inserted into the trachea similar to the balloon catheter method, but without the need to manually expand the stent. The stent’s predetermined form is a cylinder with a slightly larger diameter than the trachea, and it
returns to this form upon reaching body temperature. The catheter is then removed, and the stent is left deployed.

The nitinol tracheal stent is the primary stent in use by vets for treating tracheal collapse. This is due to the material’s superior fatigue strength, which is necessary for withstanding the typical bending movements of a dog’s neck. The high fatigue strength of nitinol is due to its shape memory properties. Being a shape memory alloy, nitinol also has a property known as super elasticity. Essentially, when nitinol is at a temperature slightly higher than its transformation temperature, it is capable of continually recovering from strains for an extended period of time. For a stress of about 30 kPa, this can be up to and over 10,000,000 cycles [11]. This is due to multiphasic nature of nitinol which causes the shape memory. At low temperatures, nitinol is in a phase known as martensite, and it can be freely bent and flexed. Once it passes the threshold of its transformation temperature, it enters the austenite phase, which causes it to return to a predetermined form. While the nitinol is in the austenite phase, any stresses applied can force it back into the martensite phase. If the temperature remains above the transformation temperature, the nitinol will return to the austenite phase and its chosen form upon the release of the applied stress.

The most recent advances in stents have occurred with drug eluting stents and biodegradable stents. Biodegradable stents are generally made of a polymer that is designed to degrade over time until it is completely removed from the body. In the case of canine tracheal stents, biodegradable stents are not feasible. This is due to the fact that the stent would need to remain in the dogs trachea for up to 15 years.

Drug eluting stents are coated in a polymer that is loaded with a drug, allowing for the drug to be released into the body as the polymer degrades. For a canine tracheal stent, the eluted drugs are used to control the body’s negative foreign body reaction. Drugs commonly utilized in these stents include Dexamethasone, Biolimus A9, Sirolimus, Paclitaxel, and Everolimus. Excluding Paclitaxel, these drugs are immunosuppressants [12]. Paclitaxel is an antiproliferative utilized specifically for preventing the growth of scar tissue. The stent’s polymer coating is loaded with one of these drugs, and the body’s natural processes slowly break down the polymer over time. As the polymer breaks down, the drug that the coating was loaded with is slowly released. Before a drug eluting stent is released to market, its polymer degradation and drug release profiles are
studied in detail, to ensure that the rate that the body is exposed to the drug is well-known and controlled.

2.2.4 Current Limitations of Stents

The major limitations of stents are based around biocompatibility. Specifically, the foreign body response caused by the immune system serves as the greatest limitation. After implantation, the most common cause of failure in tracheal stents is the growth of scar tissue, or fibrosis. Once the stent is implanted, physiological reactions, such as inflammation, occur. This inflammation further triggers a cascade of other immune responses as the stent agitates the surrounding tissue [13]. The immune system essentially tries to attack the foreign body, the stent in this case, or at least isolate it from the rest of the body to prevent any potential damage. Fibrosis, the production of a fibrotic tissue in response to a wound, is the method in which the immune system isolates the stent from the tracheal wall. This is due to the body perceiving the interaction between the tracheal wall and stent as a type of wound [14]. This fibrotic tissue is more dense than regular tissue, and can also damage the structure of underlying tissue [15]. As this fibrotic tissue grows, it pushes on the stent, applying elevating levels of pressure as more tissue is grown, eventually breaking the stent. The previously mentioned drug eluting stents are thought to be quite useful in decreasing fibrotic tissue growth. The drugs released from drug eluting stents tend to be either immunosuppressants, which slow or stop immune response, or antiproliferatives, which prevent the growth of certain tissues [16].

2.3 Biocompatibility

2.3.1 Cytotoxicity

Cytotoxicity is the property of a material that leaches toxic chemicals that are harmful to cells. If a cell is exposed to enough of a cytotoxic material it will trigger cell death or apoptosis. Implanting a cytotoxic material into a living organism can cause an area of necrosis, or tissue death, to develop around the implant [17]. Due to this it is important to consider possible cytotoxic effects when designing a device that will be implanted into an organism especially if the device is meant to remain in the body for long periods of time. It is possible that materials that are not inherently cytotoxic may still break down into cytotoxic byproducts throughout the life of an implanted device such as hip implants which can use ultra-high weight polyethylene (UHMWPE). UHMWPE is not toxic in small amounts but when a fibrotic layer of tissue grows over the implant
and isolates it from the body, the UHMWPE can build up cause cell death [18]. and However, research prior to testing ensures that this rarely occurs.

2.3.2 Granulation Tissue Growth

The development of granulation tissue is a normal bodily immune response to a wound. When a wound occurs, it is important for the body to quickly seal it to prevent excessive blood loss. Due to the need for the body to close wounds quickly, normal healthy tissue does not have time to grow as the wound clots. Instead fibroblasts quickly produce fibrin, which triggers the formation of a network of fibrin at the wound site. This signals the immune system to remove the damaged tissue through phagocytosis as new healthy tissue begins to grow. To complete the healing of the wound, the new healthy tissue is vascularized in order to reconnect it to the circulatory system and allow for normal cell life to continue [19].

Despite the growth of granulation tissue being a normal healthy response to a wound, it can also be detrimental for blood vessels, the trachea, and other open ducts if the tissue continues to build up until it completely occludes or blocks the duct. All implants trigger some form of foreign body response, which is the body’s way of protecting itself from foreign contaminants. In order to create a healthy implant, the foreign body response needs to be minimized to prevent the excessive buildup of granulation tissue [19].

2.3.3 Epithelialization

While fibrosis is tissue growth that is detrimental to implanted stents, small amounts of tissue growth on and around stents is not always bad. Epithelial tissue growth is vital to preventing stent migration and thereby increasing the longevity of an implanted stent. Epithelization is the process of growth of epithelial tissue over the stent in a layer of cells about 4-5 cells deep.

For stents, epithelization is vital to biocompatibility. One of the main problems that is addressed by increasing the stents biocompatibility is the growth of scar tissue. In growing this scar tissue, the body is attempting to isolate and attack the stent as a response to stent migration which causes irritation and inflammation. When a layer of epithelial tissue grows over the stent, it isolates the stent since the body is not equipped to break down a stent itself. Essentially, the epithelial tissue separates and protects the stent from the body, preventing the immune system
from detecting the stent, and in turn preventing the fibrotic response to it [20]. Without the fibrotic tissue growth damaging the stent, its lifespan can be greatly enhanced.

Any movement of the stent can cause injury to the trachea, which can provoke an immune response. Epithelization is able to prevent migration of the stent, thereby reducing the chances of this potential issue[21]. As long as the stent is designed to allow for epithelial exposure, it will be covered on all sides by cells. This epithelial layer that is grown over the stent immobilizes it, preventing any movement that could potentially cause damage and reducing the chances of the stent being coughed up.

2.4 Coatings

2.4.1 PLLA

PLLA, or poly-L-lactic acid, is biodegradable polyester. It is a well-known polymer for use in stent coatings, being one of the more commonly used coatings [22]. PLLA is used in both copolymer and block copolymer form with other polymers. PLLA degrades primarily by hydrolysis and is not known to produce any toxic byproducts as it degrades. Its monomer, lactic acid, is a natural product of the human body, and is subsequently turned into water and carbon dioxide by the body’s natural metabolic processes [23]. One of its downsides is that it degrades by bulk degradation, and not by surface degradation. Bulk degradation is degradation that occurs to the entire volume of the polymer at once. The chemical reactions that start at the exterior of the polymer cause chain reactions that weaken the interior of the polymer volume as well. Surface degradation, however, is when only the outmost portion of the polymer degrades. Once that portion has completely degraded, the new outermost portion degrades. Bulk degradation is often thought of as inferior to surface degradation, as bulk degradation will cause the subject polymer to lose physical integrity much sooner.

The other forms of PLLA, PDLA, and the primary form, PLA, can all be used in coatings as well. The primary difference between these forms is the physical orientation of the molecules that make them up. PLA is the common form, with PLLA and PDLA being the variations from the common form. Their mechanical properties vary widely, and they have different degradation rates. PLLA, the strongest of the three, degrades within a period of anywhere between 12 months
to more than 24 months. PDLA, with a lower modulus than PLLA, degrades in 12 to 16 months [24]. Standard PLA completely degrades within 12 months [25].

2.4.2 PGA

PGA, or poly glycolic acid, is a biodegradable polymer. PGA was not originally considered to be a useful material for implantation due to its short lifespan in the body, about 90 days. Because it hydrolyzes quickly it did not seem suitable for any load-bearing applications because it would dissolve before the body could heal properly. However, it is currently used in dissolvable sutures, which don’t need to remain in the body as long as other implanted devices, such as hip implants or stents [26].

2.4.3 PLGA

PLGA, or poly lactic co-glycolic acid, is a copolymer made up of glycolic acid and lactic acid. It has already been FDA (Food and Drug Administration) approved for a variety of uses within the body due to its biocompatible and biodegradable qualities. PLGA degrades by hydrolysis of its ester bonds. When hydrolysis occurs PLGA breaks down into its polymer components: glycolic and lactic acid. Both glycolic and lactic acid occurs naturally within and is used by the body, which makes PLGA have a minimal foreign body response. By changing the ratio of lactic acid to glycolic acid, the properties of PLGA, such as degradation rate, can be tailored to various applications, including implants and sutures [27].

2.4.4 PCL

PCL, or polycaprolactone, is a biodegradable ester that degrades by hydrolysis breaking down the ester bonds. It also has a low melting point for polymers, 60°C, which allows it to be easily formed by submerging it in water heated to temps near this for a short period of time. PCL also degrades slowly compared to other polymers, which makes it a good candidate for long-term implantation. Unfortunately, it is also resistant to adhesion, which is problematic when trying to keep an implant in place without the aid of other adhesives or sutures.

2.4.5 PEG and PEO

Polymers such as PEG (polyethylene glycol) and PEO (polyethylene oxide) can be used to coat other materials to increase their biocompatibility. PEG and PEO are the same polymer with different molecular weights, meaning they all have the same monomer subunit, but each of these
polymers has a different number of monomers attached in a chain to form one polymer [28]. PEG has been used to coat drugs to carry a hydrophobic drug in a micelle, or self-forming vesicle, of hydrophilic PEG polymers in a process called PEGylation [29].

2.4.6 Chitosan

Chitosan is a biological polymer that is derived from chitin, the primary component of crab shells. It is used in a wide variety of industries, from agriculture to medicine, due to its antifungal and antibacterial properties. A major issue of chitosan is its solvability. Traditional solvents, such as acetone, chloroform, and water, are ineffective on chitosan. Generally, a weak acid, such as acetic acid, is required to dissolve it.

Another interesting property of chitosan would be its mucoadhesion, which allows it to easily adhere to mucosal membranes. This mucoadhesion allows chitosan to be effectively used as a drug delivery method or as an implant material in environments rich with mucus. Chitosan also dissolves in acidic environments, making it an effective drug delivery method in unique environments [30].

2.5 Drugs

2.5.1 Sirolimus

Sirolimus is a drug used in drug eluting stents. It functions as an immunosuppressant and an antiproliferative, preventing tissue growth caused by immune reactions. Tissue growth caused by an immune reaction is the result of an extended chain of molecular reactions that occur in the body. Sirolimus achieves its immunosuppressant properties by inhibiting one of the early steps in this chain reaction, the activation of immune T and B cells, by targeting the protein kinase mammalian target of rapamycin (mTOR). mTOR is a central part of two protein complexes, mTORC1 and mTORC2, that directly affect cell growth and movement. Without this activation occurring, the amplification of the immune response is greatly diminished, which in turn slows or prevents the growth of fibrotic tissue produced by the immune system [31].

Sirolimus can be potentially dangerous, due to it being able to weaken the patient’s immune system. When this occurs, a simple infection can potentially become deadly. In conjunction with this, Sirolimus is also an antiproliferative, which can slow the healing of other wounds [32].
2.5.2 Everolimus

Everolimus is a derivative of Sirolimus, and as such works similarly. It acts as an immunosuppressant, slowing or stopping the immune response to an implant. It has only a very subtle chemical difference from Sirolimus, the exchange of a hydrogen atom for a 2-hydroxyethyl chain. The primary change that this causes in the drug is that it is much more soluble, and thus bioavailable, than Sirolimus [33].

The changes in Everolimus that make it different from Sirolimus also seem to decrease the overall immune suppression, reducing the risk of infections becoming deadly. This may be because the drug works on only the mTORC1, rather than both the mTORC1 and mTORC2 like Sirolimus.[34].

2.5.3 Paclitaxel

Paclitaxel is an antiproliferative drug that works by stabilizing the microtubules found within cells. Essentially, this causes the cells to be unable to progress through mitosis. After being stagnated at this point, the cells will either be forced to go through apoptosis or revert to the G phase. For tracheal stents, this prevents the cell proliferation that is caused in response to an implant [35].

Paclitaxel operates in a relatively unique way. Most drugs that operate on microtubules disassemble them, but Paclitaxel prevents them from disassembling. Paclitaxel has extremely poor solubility and tends to have a large variety of side effects ranging from nausea and tingling in the limbs to fever and female infertility. As such, doctors tend to prescribe additional drugs to manage the side effects of Paclitaxel [36].

2.6 Processing

2.6.1 Polyelectrolyte Multilayers

With more recent advances in stent design, controlled drug release has become a beneficial property for controlling the host’s immune response. As such, a method for tunable release profiling of stents known as polyelectrolyte multilayering is utilized to not only allow for the controlled elution of drugs carried by stents, but also to enhance the existing surface properties of implanted devices. This method also enables the deposition of coatings on almost any possible substrate, including stents and other devices [37], [38].
Polyelectrolyte multilayers (PEMs) are utilized to allow a device to release drugs for a controlled duration following implantation. In doing so, the foreign body immune response can be suppressed, or healing can be accelerated. To create a coating allowing for tunable drug release, the layer-by-layer deposition method of polyelectrolytes, where polyanion (negative) and polycation (positive) polymers are layered onto the device, is utilized [37], [38]. These layered polymers are sensitive to pH changes within the environment they are subjected to, and will degrade in a profile that can be measured in vitro [38]. Common drug release methods that take advantage of this pH sensitivity include pH-induced swelling, pH-induced degradation of hydrogen-bonded films, and salt-induced degradation [37].

Additionally, PEMs grant biocompatibility properties to implant surfaces. Since the PEM coating takes on the properties of the polymers utilized to develop it, antibacterial and antimicrobial properties are feasible to obtain [37], [38]. For example, chitosan, a common biocompatible coating material, can be utilized in conjunction with other polymers, such as hyaluronic acid, to bestow properties ranging from bacteria resistance and implant anchoring to cell adhesion [38], [39]. A range of compatible polymer bilayers can grant properties beyond the standard biocompatibility increases found in typical coatings.

Applying such coatings requires two primary phases. The polymers must be dissolved in appropriate solvents to become liquid or gelatin-like substances. Following coating preparation, the coating is applied to the device, in the case of stent wires it can applied either by spraying the surface with the coatings or through dip coating. This allows for simple coatings to be applied even in cases where access to expensive processing equipment is limited.
3.0 Project Strategy

3.1 Initial Client Statement

The initial client statement covered a wide array of issues presented by canine tracheal collapse and included components that were eventually removed: “The goal of this project is to research and test materials and coatings for canine tracheal stents. A successful stent will not break due to forces applied by the dog (breathing, coughing, moving, flexibility), decrease scar tissue growth around the stent, and keep the stent in place following implantation. Further, to develop an implantation method for tracheal stents that conform to a dog’s tracheal anatomy, and a testing method for said stent and implantation device.” The client's need for improved canine tracheal stents and the problems associated with existing stents served as the basis for this project. Dr. Rozanski presented an ideal price range for a newly developed stent ($1,500 or less in cost to the hospital) and encouraged the team to improve upon existing stents. Most stents utilize either a balloon or sheath catheter, so utilizing either existing deployment method allowed for ease of use.

3.2 Design Requirements (Technical)

Upon receiving the initial client statement, a collection of various objectives was compiled. Based on a hierarchy of importance, these objectives were divided into primary and secondary, and would become the overall end goals the project would work toward. Using these objectives as a base, the primary functions of the design were determined. Finally, using further information gleaned from the sponsor and the literature review, the major constraints of the project were also defined.

The primary objectives were determined to be the overall durability of the device, the ability of the device to maintain the opening of the trachea, and the biocompatibility of the device. The secondary objectives, the lower priority goals that would enhance the device, were determined to be the compatibility of the device with current delivery methods, the low level of invasiveness of the device’s implantation, and the cost of the device. These primary and secondary objectives determine the necessary enhancements to be made on the current technology. To enhance existing canine tracheal stent technology and allow for future devices to utilize the innovations made by this project, an applied coating was determined to be the best option.
3.2.1 Primary Objectives

The primary objectives of this project center on increasing the quality and length of life for the dogs that require a device to correct tracheal collapse. With respect to this need, ensuring that the device possesses the ability to successfully maintain the lumen of the reopened trachea serves as a key objective. The cross-sectional area of the trachea should be maintained as close to 100% of the original size as possible to allow for normalized respiratory action.

Typically, Yorkshire Terriers are diagnosed with high grade tracheal collapse, which requires surgical intervention and/or a device for correction, at approximately 7 years of age. As the average reported lifespan of a Yorkshire Terrier is 14 years, the device should viably be able to survive for the remainder of the dog’s lifespan, necessitating the ability of the device to survive in vivo for up to 7 years as a core objective. The device must not consequently fail due to fracture, the development of scar tissue, or the movement of the device from its deployment location. As such, ensuring that the device has a high enough fatigue strength and load strength to survive for 7 years. The strength and number of cycles the device needs to survive depends on the overall design of the device. For example, a human tracheal stent needs to survive 3.3 kPa in tension and 5.25 kPa in compression on a regular basis, and the standard nitinol stent can survive for 10,000,000+ cycles of 33 kPa \[11, 40\]. Depending on the design, the values would need to be converted for use in dogs. Should the device break, invasive surgery would likely be required, which would lower the dog’s quality of life.

The final primary objective is that the device must be biocompatible with the dog. For this application, it means that the device should interact favorably with the tracheal environment. The general statement with regard to biocompatibility is that the device should not be cytotoxic, or toxic to the cells around it. A major aspect of this is how tissue grows around the device. A layer of epithelial tissue should grow over the device, around 4-5 layers of cells, but under no circumstances can there be growth of granulation or scar tissue. If scar tissue were to develop near the device, it has the potential to cause device failure. The healthy growth of epithelial tissue would also enhance the ability of the device to withstand movement from its original position. The device should also not cause general inflammation of the surrounding tissue after implantation. This would only lead to an increased likelihood of scar tissue development. Finally, the body should not cause the enhanced degradation of the device. If the environment the device was placed in was
to start degrading it, then the lifespan of the device would decrease drastically, decreasing the overall fatigue and load strength.

3.2.2 Secondary Objectives

The secondary objectives are more varied than the primary objectives. The first secondary objective relates more closely to the primary objectives than the other two. It is that the implantation of the device should be as minimally invasive as possible. This is to enhance the overall quality of life of the dog by not forcing them to go through a recovery period. The reason why this falls into secondary objectives and not primary is that if the implantation of the device is invasive, but it also essentially cures the tracheal collapse and there is no need for further action, then it would be superior to a minimally invasive device implantation that had to be repeated multiple times. The second secondary objective is that the device be compatible with current implantation methods. For something like a stent, it would mean implantation by catheter, or for something like extraluminal rings it would be surgery. This is simply to allow the use of the new device as soon as possible, without needing to teach vets a new implantation method. The last secondary objective is that it cost, at most, $1500. The current standard costs around $1,100, but if the new device is superior to the standard then a higher price won’t be an issue. The overall budget of the project for procuring materials, however, is only $650, so it will ideally be less expensive. As the subject of this project is adding to the current technology, and not replacing it, the ideal max price would be $400, as this adds with the current cost of the stent to the total of $1500.

3.2.3 Functions

The design functions of the device are what the device must accomplish through its use. The first and most important function of the device is that it will reopen the collapsed trachea of a Yorkshire Terrier suffering from tracheal collapse. This is the fundamental function of the device, and the following functions are to ensure that the device can accomplish this function. The second function of the device is that will resist migration from its original location of placement. If the device were to move, then there is a possibility that the dog would be able to cough it up or experience tracheal tissue damage. The third function of the device is that it will resist fatigue caused by the movement of the trachea during normal motion. Should the device be unable to resist this fatigue, it would break and potentially cause more damage to the dog. Regardless, the device
would need to be removed and replaced before it was able to function correctly. The fourth and final function of the device is that it will be biocompatible, preventing the growth of granulation tissue around the device and enhancing the growth of healthy epithelial tissue. As granulation tissue grows, it would put excess force on the device, likely causing early fracture, and the growth of healthy epithelial tissue would enhance both the adhesion of the device to the tracheal and the overall biocompatibility of the device.

3.2.4 Constraints

For the design of this device to treat dogs with canine tracheal collapse, there are several constraints that limit the project. Most obviously, there is limitation of budget. The device can cost up to $1500, with the developed coating costing up to $400, and the design team only has $650 to work with. There is also the limitation of testing material and equipment. Any material for testing will need to bought using the previously mentioned budget, and the number of cadaveric tracheas that the team has access to is limited by the number of donations that Tufts receives. The equipment the team has access to is limited to what can be accessed within the laboratory and additionally on the WPI campus. Finally, there is the constraint of time. The project must be complete by the end of March or early April to comply with graduation requirements.

3.3 Design Requirements (Standards)

Several industry standards will need to be met in order to create a safe product. These standards also fall parallel with the overall project objectives. For this project, there are four standards set by the International Standards Organization (ISO) and the American Society for Testing and Materials (ASTM) that will need to be met.

3.3.1 Industry Standards

The first ISO standard that needs to be met, ISO 10993-11:2017, which tests for toxicity in medical devices, is directly correlated with one of the primary objectives of this project, to make the device biocompatible. Meeting this standard ensures that any device that is implanted in a canine does not intrinsically cause harm by killing off the surrounding cells due to toxicity. The second ISO standard involved in this project is ISO 10993-1:2009 which is related to ISO 10993-11:2017. This ISO standard also includes testing or auxiliary research to indicate that the device will not be harmful based on the material, manufacturing processes, geometric shape, contact area
with the body or its fluids, and its sterilization process. All of these standards were set in place to ensure that when a medical device is implanted, it has the highest possibility of not being harmful to its host. The last ISO standard that will need to be met by this project is ISO 11737-2:2009, which involves being able to sterilize a device before it is implanted. The inclusion of this ISO standard is based on the request of the sponsor of this project rather than necessity since the moment the device is opened to be implanted it would no longer be sterile. The one ASTM standard that will also need to be met for this project is ASTM F2063-12, which standardizes the use of nitinol for medical devices and implants.

3.4 Revised Client Statement

After determining that the project should not concentrate on developing a new stent, it was refocused onto stent coatings. Instead, the client statement was revised to focus on determining a materials-based solution for existing stent biocompatibility and adhesion issues. The statement reads as follows: “The goal of this project is to develop and test coatings for canine tracheal stents. A successful stent coating will decrease scar tissue growth around the stent, keep the stent in place following implantation, and be applicable to stents that are currently available to surgeons.” The overall goal is to utilize coatings and processing techniques to improve the stent’s performance and longevity within the patient. The coating must be noncytotoxic, allow for positive cell adhesion to stay in place, and prevent the growth of excess scar tissue within the trachea. The developed coating must assist the stent in lasting for 7 years after implantation based on the average age of diagnosis (7 years) and the lifespan of Yorkshire Terriers (14 years). Overall, the coating must reduce the negative immune system response to the stent, assist with adhesion, and be compatible with the cells of the trachea.

3.5 Management Approach

3.5.1 Work Breakdown

Throughout A Term several important milestones have been met. To start this project, research was conducted in fields related to medical devices that treat tracheal collapse, the properties that make a device biocompatible, possible materials and coatings that could be used, and anatomical research on canines. The research on materials and coatings was then used to create
a list of possible options that could be used to create a device to assist a stent’s functions within a canine trachea.

B Term served as the starting point for testing the list of suitable materials and coatings to establish which materials and coatings can cause cytotoxic responses, tissue irritation, and granular tissue buildup. Additionally, the project methods were refined to allow for additional testing and narrow down the candidate materials to determine the best final candidate.

During C Term, the materials underwent additional verification testing to determine the final candidate for in-depth testing. Following the completion of the verification tests, the data underwent statistical analysis. This validation enabled the team to determine the successfulness of the project and allowed for the completion of a third draft of the report.

The project candidate testing was finalized in the beginning of D-term. The report was finalized in D term with a deadline of April 26, 2018. The results of the project were presented on April 20, 2018 for WPI’s project presentation day. Following the completion of both requirements, the project was considered complete.

3.5.2 Gantt Charts

![Gantt Chart]

*Figure 1: Fall Semester Work Breakdown*
3.5.3 Financial Considerations

The project team received a budget of $750. Access to the laboratory space cost $100 and granted access to basic cell culture laboratory supplies. The remainder of the budget purchased supplies for developing and testing stent coatings. Table 1 below displays the budget tracking sheet.

![Task Breakdown Diagram]

*Figure 2: Spring Semester Work Breakdown*

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<table>
<thead>
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<td>Cell Adhesion Testing</td>
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<td>Cytotoxicity Testing</td>
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<th>D-Term</th>
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Table 1: Budget Tracking Sheet for Project Items

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<tr>
<th>Component</th>
<th>Cost</th>
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<tbody>
<tr>
<td>Lab space and basic supplies (acetone, chloroform, 5% acetic acid)</td>
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<tr>
<td>Medical grade nitinol wire (72 in.)</td>
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<tr>
<td>Chitosan (50g)</td>
<td>$68.10</td>
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<tr>
<td>PCL (Donated by Professor Marsha Rolle's Laboratory)</td>
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<tr>
<td>PLLA (3g)</td>
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</tr>
<tr>
<td>Total</td>
<td>$323.89</td>
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</table>
4.0 Design Process

4.1 Needs Analysis

4.1.1 Needs Criteria

The primary need fulfilled by this project is extending the lifespan of and enhancing the quality of life for Yorkshire Terriers that are suffering from advanced stage tracheal collapse. Tracheal stents, the primary current solution, tend to last for around 2 years, while Yorkshire Terriers are usually diagnosed with the disease at the age of 7 and live to 14 years of age. The alternative treatment method, extraluminal tracheal rings, is highly invasive and can lead to further complications. The tracheal stent’s short lifespan and lack adhesion and integration into surrounding tissue creates the need for a solution that is both more biocompatible and durable.

4.1.2 Functions and Specifications

The objectives of this project, as previously mentioned, are durability, maintaining the tracheal opening, biocompatibility, compatibility of the device with current delivery methods, minimal invasiveness of the device, and the overall cost. These objectives were refined and weighed against one another to determine which were most critical for a stent to fulfill. The results of this comparison can be examined in Table 2 below.
The primary function of a device that corrects tracheal collapse in Yorkshire Terriers is to open and maintain the cross-sectional area of the trachea as close to 100% of the original area as possible. This would improve the overall breathing capability of a patient suffering from the disease. A coating applied to a stent must assist the stent in improving any mechanical and biological functions that are necessary in correcting tracheal collapse. The coating must additionally fulfill several other criteria, including resisting the forces that the coating would be subjected to in the dog’s trachea for approximately 7 years, resisting migration following the
deployment of the stent, and promoting the growth of healthy epithelial tissue while inhibiting the growth of granulation tissue.

The human trachea is exposed to forces 3.3 kPa in tension and 5.25 kPa in compression [40]. A dog’s neck is also able to rotate with more degrees of freedom than a human’s, so the values may be higher. However, there have been studies showing that the current gold standard for advanced tracheal collapse treatment, the nitinol tracheal stent, is capable of surviving 10,000,000+ cycles of loading at 33 kPa [11].

The overall migration of any device that would be placed in the dog's trachea to correct collapse should be minimized. Any migration of the device increases the likelihood of irritation and may cause the dog to cough and dislodge the stent entirely. Increasing the radius of the stent to be slightly larger than that of the trachea reduces this risk [3]. However, dislodgement can still occur due to poor adhesion to the wall. As such, the coating should assist the stent in maintaining its deployed position and reduce irritation in the surrounding tissue.

Additionally, the last function of the coating is to increase the biocompatibility of the stent. For this project, this primarily means that the device should enhance the growth of epithelial cells over the stent while inhibiting the growth of granulation tissue. The growth of epithelial cells reduces the foreign body response from the surrounding tracheal tissue. This helps to prevent the growth of granulation tissue, which can fracture stents placed in the trachea, block the airway, or cause tracheal paralysis as it builds up over time. The growth of epithelial tissue also enhances the adhesion of the device to the tracheal wall. A layer about 4-5 cells thick would increase the overall adhesion of the device to the tracheal wall, decreasing the likelihood of dislodgment, and reducing the foreign body response.

Using the current gold standard as a base, the functions previously listed were enhanced by utilizing a designed and tested coating technology. As such, the coating itself does not need to provide these functions to the stent, granted that stent already produces the desired results. The coating itself doesn’t need to produce an increase in fatigue strength, instead it needs to be able to resist at least the same forces as the device. For increasing the lifetime, the coating should protect the device from bodily processes that would weaken its other properties, such as fatigue strength and biocompatibility.
4.2 Alternative Designs

4.2.1 Non-Coating Alternatives

The direction that was chosen for this project was a coating for a tracheal stent, but there were similar options that could have been utilized. Specifically, the stents could have either undergone surface processing or anodizing. These are both processes that involved the direct modification of the stent’s surface, rather than the addition of new materials in a layer over the stent.

Surface processing is the modification of the surface of a stent to add ridges or grooves to the surface [41]. This increases the friction between the stent and the trachea, increasing the stent’s ability to grip the trachea. Grooves and ridges also provide places for cells to grow into, which will allow the eventual growth of tracheal tissue over the stent. Surface processing is generally a chemical or mechanical process, where either etching chemicals, milling implements, or lasers are used to remove material from the surface of the stent.

Anodization is the oxidizing of the surface of the stent, converting it from the pure metal state to the oxide state [42]. Oxides are much more resistant to corrosion than their metal counterparts, as they are technically already corroded through oxidization. This would extend the overall lifetime of the stent, as it normally corroded over time in the body. Oxides also provide a good base to hold drugs when layered with a polymer coating, and drugs could have been used to manipulate other properties between the stent and the trachea, such as cough suppressing and cell growth. Anodization is accomplished through the application of an electrical current to the stent while the stent is exposed to specific chemical reactants.

These non-coating alternatives were determined to be inefficient for the purposes of this project. Surface processing requires machining elements that were not available or required advanced training on said machines. Anodization was determined to not add or increase the properties that were being examined by this project, specifically biocompatibility. So, these options were ruled out, and coating options were examined.

4.2.2 Coating Alternatives

Coatings were determined to be the optimal design process for the project. This conclusion was reached due to the limitations that were on the project for time, resources, and skills. After that
decision was made, however, other options were considered. Most notably, the coating to utilize and the coating method.

To determine the coating to use, there were three options that had been found: A layered coating, a multistage coating, and a tracheal hardening coating. The layered coating is the simplest of the three, as it can be made with a single polymeric material [43]. This also makes it the most versatile. The layered stent can be made of multiple materials, it can be loaded with drugs, and it can include one to multiple layers. In the case of a drug eluting layered coating, the drugs would be loaded in the lower layers, and the upper layers would be used as the controlled release gate for the drug. The multistage release coating is like the layered coating, except that it is always a drug eluting coating and will always be made of more than one material [44]. The drugs will be within different layers of the coatings, and their control gates would be different polymers. This ensures that certain drugs are released quickly and early in the stents lifetime, while other drugs are released slowly and later in the stents lifetime. Depending on the polymers used, there can also be very specific triggers used to release the drugs, such as temperature or acidity. The third type of coating, the tracheal hardening coating, is purely theoretical on the part of this project. That is, it currently does not exist. The tracheal hardening stent would be a specific subclass of the previous coatings, one designed to reharden the softened trachea instead of delaying the eventual collapse. Of these three types of coatings, it was decided that the layered coating would be used for this project. This was decided as it was the most versatile, but also the most cost effective. Drugs were determined to be beyond the capabilities and budget of this project, which removed the other two types of coatings.

After choosing what kind of coating was needed, the method of applying said coating needed to be decided upon. Research pointed to three major coating methods: Dip Coating, Spray Coating, and Electrospinning. Dip coating is the simplest of the three methods. The stent is dipped in the dissolved polymer, and then the coating is allowed to dry [45]. Spray coating is similar except the coating is applied using a spraying device. This allows for much more control over the coating thickness [46]. Finally, electrospinning is the release of a small stream of polymer, depositing it in a random spinning pattern that fills out into a full coating as it is applied, and it dries [45]. It was decided that the dip coating method would be used for this project. The dip
coating method required no technology that needed to be acquired or learned, thus providing the most efficient option energy wise and cost wise.

Finally, the material that was to be used for the coating needed to be decided on. In an effort to conserve the project budget, three polymers were chosen to be tested. These polymers were PLLA, PCL, and Chitosan. PLLA is a common biocompatible polymer, used in numerous medical devices and the primary material used in 3D printing [47]. PCL is very similar to PLLA but tends to have a longer lifetime [48]. Finally, Chitosan is a biopolymer that is derived from the chitin in crab shells [49]. Research has found that there are specifically useful properties in chitosan, such as being biocompatible and generally mucoadhesive [49]. These three polymers were chosen to be tested against each other, and the polymer that would prove to be the best would be chosen as the final material for the polymer coating.

4.3 Testing Methods

The needs outlined in Chapter 4.1 serve as the basis for the concept designs for the tracheal stent. These designs fulfill one or more of these individual needs: applied force, migration prevention, and biocompatibility.

4.3.1 Surface Polymer Coating

The surface of the stent can be coated with a polymer to assist in adhesion to the tracheal wall. These modifications would allow for the stent to “grip” the tracheal wall itself, prevent migration, and increase the stent’s overall biocompatibility. The major options for polymer coatings that we will explore are poly-l-lactic acid (PLLA), polycaprolactone (PCL), and chitosan [47]-[49]. As for the method of coating, the team chose to use the dip coating method. The polymers and method were chosen primarily based on risk and cost. That is, after they met the requirements for biocompatibility. Other possible polymers were explored in the literature review, but the alternate methods of coating were spray coating, electrospinning, and polyelectrolyte multilayers. However, spray coating and electrospinning call for specialized equipment that was not accessible for this project, and polyelectrolyte multilayers require the polymers to have alternating positive and negative charges. Thus, we turned to dip coating, which can be done with forceps and an incubator.
The general method of dip coating is always the same: dissolve the polymer in a solvent, dip the subject being coated in the polymer solution, then allow the coating to dry. There are slight variations depending on the polymer, such as what solvent is used and if there is an additional dip following the drying of the coating. This dip in an additional fluid is used to ensure the solvent is completely removed from the drying polymer. The second dipping substance is generally a chemical that reacts with the solvent, pulling any remaining solvent out of the polymer coating. In general, any coating is at least washed with deionized (DI) water just to be careful. The solvents used in these methods are Chloroform for PLLA, Acetone for PCL, and 5% Acetic Acid (vinegar) for chitosan [47]-[49]. The PLLA and PCL were washed with DI water following drying, and the chitosan was dipped in a mixture of household ammonia and 75% isopropanol ethanol. Once the samples were coated and dried, they were imaged using a ZEISS AxioCam ERC 5s camera on a ZEISS Primo Vert microscope. The magnification was 20x to get a clear view of the sample and coating, and the phase should be Ph1/0.4. The general process is displayed below, in Figure 3.

![Figure 3: The Simplified Polymer Dip Coating Process](image)

4.3.2 Cytotoxicity Testing

The inflammatory response to foreign bodies in the trachea results in the growth of scar tissue. The use of a coating in order to reduce inflammation and tissue irritation by increasing tissue adhesion would greatly improve the longevity of an implanted stent. However, while increasing tissue adhesion is important it is also critical to assess whether the material or coating has any possible cytotoxic properties associated with it.
Any coating used must be noncytotoxic and allow for cell adhesion. The feasibility testing for this coating was conducted by using an MTT assay which is an ISO standard for testing cytotoxicity. This assay works by first seeding a known number of cells into various wells of a 96 well plate and then adding tetrazolium dye to each well. The dye is then reduced into formazan by living cells which is a non-soluble byproduct of regular metabolic cell activity and has a purple color. After running the 96 well late through a spectrometer it is possible to measure the amount of purple dye present and correlate this with the amount of cell life present in each well. This data is then graphed, and a line of best fit is calculated to create a standard curve which allows for unknown values of cell life to be calculated. After the standard curve has been determined each material can be tested for cytotoxic properties by first creating leachates by placing a small coated stent sample into cell culture media and allowing any possible toxic chemicals to leach into the media. These leachates are then removed and used to feed cells in a 96 well plate that were seeded at 40,000 cells per well. After allowing the cells to incubate for 48 hours the tetrazolium dye was added allowed to incubate for another four hours. This plate is then run through a spectrometer and the absorbance values recorded are then compared to the known values in the standard curve to assess if any cytotoxic chemicals affected the cells.

4.3.3 Force Testing

As Chapter 4.1.2 specifies, the stent must be able to withstand approximately 35,000,000 loading cycles at 33 kPa over its lifetime. This is based on human data that was adapted to the more flexible dog trachea by increasing the necessary forces and loading cycles. The shape and structure of the stent allows for it to withstand more force. While this aspect of stent design is beyond the scope of this project, it is important to consider using flexible, fatigue-resistant shape-memory materials as the structural basis for these stents. These materials are the gold standard for the canine tracheal stenting market, and any coating that is designed must be compatible with these materials, specifically nitinol. A dip coating for a nitinol stent must be able to also withstand cyclical loading with the same forces without degrading to be fully compatible with the stent.

To test the loading capabilities of a coating or material, two tests can be utilized: mechanical cyclic loading via a testing machine or loading using a simulated trachea. The mechanical cyclic loading would utilize the Instron 5544 and the corresponding Bluehill software to run long-term testing procedures for the required number of cycles and force level (35,000,000
at 33 kPa, or 40,000,000 when adjusted for any error). This data would be run through an analysis code in MATLAB to determine if there was any indication of failure or changes in mechanical loading response during the testing phase itself. The material or coated stent would then be physically examined to search for any defects or degradations on the surface. It would be considered a success if still intact and if the data did not show any indications of failure.

Load testing using a simulated trachea would allow for the stent and coating to undergo the loading cycles while conforming to a trachea-like tube while testing. This type of testing would instead simulate the movement of the dog’s neck and trachea based on the ability for the dog to load its trachea. The flexibility data would likely be collected through examining cadaveric specimens for degrees of freedom in the neck and by utilizing observations from Tufts veterinarians on the number of times per day that dogs move their neck to the degree that shape change in the trachea would occur. While this would more realistically simulate the forces the stent would undergo, the apparatus would be less uniform and accurate regarding force levels and would require extensive testing prior to any testing on the stent and coating itself, since it must be constructed from scratch. The limited timeframe to complete testing makes designing and testing such a device difficult and less than ideal, making the traditional Instron testing the more favorable option. A basic 3-point bending test apparatus is displayed in Figure 4 below.
The feasibility testing itself would consist of 3-point bending testing. The 3-point bending test would position the expanded stent horizontally between two support spans while the Instron will use a third wedge to cyclically load the device in the center. The cycling would require approximately 35,000,000 cycles with 33kPa to best replicate the forces the stent will experience, which is not feasible with time and equipment restraints. Instead, lower forces and cycle lengths will be utilized. In both types of testing, the stent could be examined using an electron microscope both prior to and after testing, allowing for the microstructures of the material or coating to be validated. In a successful test, the material or coating will not fatigue, as it would inhibit the performance of the stent on the subject.

During initial testing, individual coated and uncoated wires will be examined using 3-point bending testing. This will allow for the examination of the property changes of individual wires, which will then compose the whole coated stent as a system.

4.3.4 Validation of Methods

The polymer coating method was tested first for chitosan, because it was the cheapest of the three polymers, then PCL, as it was dissolving in the acetone, and finally the PLLA, which failed to dissolve in acetone and thus required chloroform. All three polymers successfully managed to coat the nitinol wire samples, albeit with varying successes.
The chitosan solution was 2 w% chitosan in 5% acetic acid. The solution was prepared over one hour and thirty minutes, being stirred at 40°C. The samples of nitinol wire were 2 inches in length, and their diameter was 0.006 inches (152.4 micrometers). The solution ended up being very viscous, similar to the consistency of honey. For the validation images, we were required to look for the errors in the coatings. Over most of the length of the samples, the coating is even and difficult to distinguish from the wire. However, there are small areas where the coating beads or appears to splinter, and it is those areas that we looked for to validate the coating method. As can be seen above in Figure 5, all four chitosan samples showed at least one small error in the coating that allowed us to recognize the coating.
The PCL solution was 5 w% in pure acetone. The polymer was incubated at 40°C in the acetone for around 24 hours, but most likely would have been fully dissolved by 6 hours. As acetone evaporates readily, the vial that the polymer was dissolved in was sealed with parafilm. The final solution was of a similar consistency to water. The nitinol wire samples were the same as with chitosan, 2 inches in length and 0.006 inches in diameter. As can be seen in Figure 6, the PCL coated samples provided much more obvious inconstancies than the chitosan. The more obvious inconstancies show a thicker coating, which can be good, but a thicker coating also could cause issues in testing but impacting the potential for mechanical bending.
The PLLA solution was 5 w% in pure chloroform. The PLLA was first attempted to be dissolved in acetone but had not dissolved after almost a week of incubating at 40°C. In chloroform at 40°C, the PLLA dissolved within 24 hours. As with the PCL, this could probably be limited to 6 hours and it would still be completely dissolved. The final solution was more viscous than the PCL solution, but less viscous than the chitosan solution. The nitinol wire samples were the same size as the other polymers, 2 inches in length and 0.006 inches in diameter. As can be seen in Figure 7, PLLA had the most obvious coating of all three polymers. The coating was visible with the naked eye in some cases. Looking at the samples on the right, one can clearly recognize the line between the wire sample and the polymer coating.

For all three of the polymers, multiple samples were prepared. This was to allow the other tests, the MTT assay and the mechanical testing, to be validated. The validation of the testing to
be conducted on the Instron 5544 used a sample the selected wire, a piece of Nitinol SEA Black Oxide wire with a diameter of 0.006 inches and a length of roughly 2 inches. The selected strain rate and maximum displacement of the three-point bending test were 310 mm/min, and roughly 12 mm respectively. The testing was planned to take place for a duration of 1,000 cycles while recording the force and displacement undergone by the wire. Figure 8 and Figure 9 below display the testing prior to loading and during loading respectively.

Figure 8: The 3-Point Bending Test Prior to Loading the Wire
The wire was able to be bent by the Instron without rolling due to a pair duct tape “grips” centering the wire on the support span. During the initial test, the wire was loosely adhered to the actuator, and in the secondary test the wire was not adhered. The results of the tests can be observed below in Figure 10 and Figure 11 below.

*Figure 9: The Wire During Bending*
The first test failed after approximately 250 cycles, while the second test failed after approximately 300 cycles. In both cases failure occurred due to the wire slipping from the grips. While the data in test one appears to show observable force behavior, both tests produced data below the Instron’s resolution due to the load cell of the Instron 5544 not being designed for loading forces under 20N. Utilizing theoretical calculations was selected as an alternative to
physical mechanical testing due to the lack of an available load cell capable of detecting small forces.

4.4 Final Design Selection

Based on the weighted project objectives matrix, the alternative designs can be compared against one another following testing. These tests, as described above, are coating integrity, cellular adhesion, cytotoxicity, and mechanical force testing. Additionally, secondary considerations are applied to coating cost and ease of application, as they are relevant in the clinical setting due to budget and equipment requirements for coating a stent. These tests each correspond to an objective, with fatigue resistance examined with mechanical force testing, adhesion of the stent coating examined with adhesion testing, biocompatibility examined with a cytotoxicity test, and coating integrity through examination under a microscope. The example weighted design matrix can be seen below in Table 3.

Table 3: Blank Weighted Design Matrix for Candidate Materials

<table>
<thead>
<tr>
<th></th>
<th>Bare Wire</th>
<th>Chitosan</th>
<th>PCL</th>
<th>PLLA</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coating Consistency</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>Manufacturing Concerns</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Mechanical Integrity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>Non-Cytotoxic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.5</td>
</tr>
<tr>
<td>Cell Adhesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>Cost</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
</tr>
</tbody>
</table>

With these objectives outlined and weighted, comparative testing on each alternative design can be performed, allowing for the best coating option to be verified with collected data. This data was scored based on the results of each individual test. The total score determined the most viable coating for use within canine tracheal stents.
5.0 Design Verification

5.1 Coating Results

After validating the testing methods, they were utilized on the samples that would later undergo the other testing protocols. All three materials produced a coating using the designed dip coating method, as seen in Figure 5, Figure 6, and Figure 7. After discussing and determining the needs of the other tests, it was determined that at least seven samples of each coated wire were necessary to successfully carry out the experimental methods. To factor in any errors that may have occurred, such as test failures or errors, an additional three samples for each polymer coating were made, bringing the total number of samples for each material up to ten.

![Figure 12: Materials Pre-Dissolution (a) Chitosan b) PCL c) PLLA]

![Figure 13: Materials Post-Dissolution (a) Chitosan b) PCL c) PLLA]
The three materials, chitosan, PCL, and PLLA, can be seen in their raw form in Figure 12 and in their dissolved form in Figure 13. The chitosan, a flaky, white powder in its raw form (Figure 12a), becomes a yellow tinted fluid when mixed with vinegar at 40°C for two hours (Figure 13a). The chitosan mixture is viscous, even when the solution is only made with 2 wt% chitosan. Even pushing the wt% up to 5% is enough to produce a fluid with such a high viscosity that it is nearly solid. The PCL, which is white pellets in its raw form (Figure 12b), becomes a clear fluid when dissolved in acetone at 40°C for 24 hours (Figure 13b). At 5 wt% PCL, the solution is fluid, which does not change when the wt% goes as high as 15%. The PCL also does not dissolve completely on its own, instead becoming a denser fluid in the acetone. 5 min of agitation, such as simply shaking the vial, followed by several hours of rest at 40°C is enough to completely dissolve the PCL. The PLLA, which is opaque crystals in its raw form (Figure 12c), becomes a clear fluid when dissolved in chloroform at 40°C for 24 hours (Figure 13c). The PLLA solution is the most viscous of the three at its chosen concentration, 5 wt%. Chloroform was required, as the PLLA did not dissolve in acetone after several days of exposure at 40°C.
Each sample was dipped into its polymer solution for two minutes (Figure 14), and the chitosan wire was additionally dipped into a fixing solution of ethanol and ammonia for five minutes. Only the Chitosan required a fixing solution (Figure 15) to create its coating, but the PCL and PLLA coatings used a wash of DI water to ensure that solvent wasn’t left behind on the coating.
For the drying period, it was found during the validation testing that the binder clips holding the wires did not balance well on the vials. If the clips fell over during the drying period, it introduces additional errors in the coating. To resolve this issue, a holder for the vials and binder clips was designed using the PTC Creo CAD software and printed using PLA on a 3D printer (Figure 16a). 10 of these holders were printed so that a full set of samples could be made at once. The risk of imbalance wasn’t solved, but greatly reduced by the holders. The holders held the vials and the binder clips for the 24-hour drying period (Figure 16b).

The imaging process remained the same for the candidate testing as that of the validation testing. A ZEISS AxioCam ERc 5s camera on a ZEISS Primo Vert microscope was used to take the images, and its default software was used to process the images and add 50 micrometer scale bars. The magnification used was 20x, and the phase was set to Ph1/0.4. The only difference between the imaging of the validation testing and the candidate testing is that the wire samples were left in their binder clips during the candidate testing (Figure 17). The samples were difficult to remove when placed in the dish alone, and this prevented damage to the samples when trying to remove them from the dish.
Figure 18: Deposition of Wire into Vial, and Removal of Holder

After imaging, the coated wire sample was unclipped from the binder clip and deposited into the vial. To help maintain some degree of sterility, the vial holder shaft detaches from the base to allow easy removal of the vial (Figure 18).
The success of the wire coating was determined qualitatively rather than quantitatively. The coating was successful if there was evidence found of a coating, but they were considered more successful if they were more evenly-distributed or thicker. The chitosan samples (Figure 19) all showed evidence of coating, so they were considered successful coatings. There were some samples that were considered more successful due to a more even coating (Figure 19a, b, e, g, h, j). An even coating was considered better because an uneven coating carries the risk of exposing portions of the sample while covering others. Conversely, there were some that were considered less successful due to unevenness of the coating (Figure 19c, d, f, i). In general, the issue that the chitosan samples had was evenness. Despite an even coating seeming to be applied after the initial dip, the coating would later prove to be uneven following the fixing solution bath and imaging. In some cases, the fixing solution turned the chitosan coating quite opaque, and it could easily be seen that the polymer coating beaded along the length of the wire.
For the PCL samples (Figure 20), the overall coatings were extremely thin. This does not speak to the quality of the coating, as they were difficult to see using the power microscope available. It has been decided that for this project, the PCL samples are to be considered poorly coated. The PCL samples did not show much evidence at all of coating initially, and the images in Figure 20 needed to be taken after an additional coating of PCL was applied. The most apparent coatings can be seen in Figure 19a, d, e, f, h, and i. In all of these except Figure 19f, the coating appears in the image as a rough, uneven quality along the surface of the wire. Observing the wires during the dip process, there did not appear to be any coating applied after dipping, so most of the polymer dripped off when the wire was removed from the solution.
Overall, the PLLA samples (Figure 21) provided the most successful coatings in terms of adhesion to the wire. In fact, they provided samples that adhered so successfully that the coatings in some cases are too thick. Too thick of a coating could theoretically cause issues in the deployment of the coated stent. For this project, however, these coatings were considered successes. Very thick, very even coatings can be seen in Figure 21f, g, and i. Observing the samples during the coating process, the coatings could be visibly seen as the samples were transferred from the solution to their vials. In cases where the sample was tipped over and it bumped into the side of the vial, the PLLA dried the sample to the vial wall.
A summary of the coated wire diameters and their corresponding coating thicknesses can be found in Table 4 and in Figure 22. The measurements were made using ImageJ software, and then the final values were calculated using Excel. Each polymer was 14 samples measured, each with one image taken and four measurements per image. After measuring the coatings, the PLLA samples had the highest ability to coat the nitinol wire. As a contrast, the PCL samples had the lowest ability to coat the wire. Between the two materials lies chitosan, whose coating leans towards PCL rather than PLLA.
5.2 Manufacturing Concerns

The difficulty of coating is largely dependent on the ability of the material to coat and the required safety equipment for creating the coating. The ability of the materials to coat can be seen in Table 4 and Figure 22. To summarize which polymers coated better, PLLA created the thickest coatings and PCL created the thinnest coatings. The safety of creating a coating is primarily a concern when using substances such as chloroform, as a fume hood is required, but applies to any solvent that produces unsafe fumes. However, the use of the fume hood does not maintain the sterility of the samples. The superior option in this would have been to use a sterile glovebox. As PLLA requires chloroform, it would thus be considered the most dangerous, while Chitosan is the least dangerous as it only requires vinegar. PCL comes in the middle with acetone as it's solvent. The coatings will be ranked from most to least safe based on safety concerns for preparing the coating, but also based on the ease of applying the coating to the stent. Since all candidates are dip coatings, the only difference in this regard will be the necessary number of repeated coatings required.

5.3 Mechanical Testing Results

Examining the mechanical property alterations that result from adding a coating to the individual stent wires allows for the viability of each coating to be examined. In most circumstances, where a mechanical testing apparatus of proper loading limits can be utilized, 3-point bending testing provides data corresponding to the changes in force and stress corresponding to displacement and strain. However, initial testing of the super elastic wire presented forces below the Instron 5544’s resolution, in data that could not be used. With this limitation in mind, a theory-based approach was taken to evaluate the effect that coatings have on the mechanics of the stent wire, and by extension the stent itself.

Utilizing bending theory principles as a basis for these theoretical calculations, assumptions are made regarding the uncoated and coated wires. The wires are all assumed to be of equal length and wire radius, the coated wires are assumed to be cylindrical composites, and the radii throughout the coated and uncoated wires are assumed to be uniform throughout. The wires undergo 3-point bending, where a force is applied at the midpoint of the wire length while the wire is resting on supports. Figure 23 and Figure 24 below display the setup of the coated wires and the bending test respectively.
The general 3-point bending equations and the area moment of inertias of solid and hollow cylinders largely serve as the basis for the comparison of the coated and uncoated wires. These will be utilized to further derive a relationship between the mechanical properties of the uncoated and coated wires. The equations utilized are as follows:

\[
\frac{M}{I} = \frac{\sigma}{r}
\]

\[
l_w = \frac{\pi}{4} * r_w^4
\]

\[
l_c = \frac{\pi}{4} * (r_T^4 - r_w^4)
\]

\[
l_T = l_w + l_c
\]
Where the variables utilized are:

- $M =$ bending moment (N*m)
- $I_w =$ area moment of inertia of the wire (mm$^4$)
- $I_c =$ area moment of inertia of the coating (mm$^4$)
- $I_T =$ total area moment of inertia of the wire and coating (mm$^4$)
- $\sigma =$ applied stress (MPa)
- $r_w =$ radius of the wire (mm)
- $r_T =$ radius of the coated wire (mm)

In theory, these values are utilized to calculate the relationship between the coated and uncoated samples. However, due to the composite nature of the uncoated wires, the modulus-weighted area moments of inertia will be utilized. The calculations for these values are identical to their unweighted counterparts except for multiplying each component by a corresponding elastic modulus (E). The modulus-weighted equations are as follows:

\[
I_{weighted\ w} = E_w * I_w
\]
\[
I_{weighted\ c} = E_c * I_c
\]
\[
I_{weighted\ T} = I_{weighted\ w} + I_{weighted\ c}
\]

Where the $I_{weighted}$ variables correspond to the composite or its individual components. The elastic moduli utilized were based on a literature search and approximations based on value ranges. Table 5 below lists the elastic modulus values utilized for the superelastic nitinol wire and the three coatings.

<table>
<thead>
<tr>
<th>Material</th>
<th>Elastic Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superelastic Nitinol Wire [50]</td>
<td>58,000</td>
</tr>
<tr>
<td>Chitosan [51]</td>
<td>6.3</td>
</tr>
<tr>
<td>PLLA [52], [53]</td>
<td>1,000</td>
</tr>
<tr>
<td>PCL [54]</td>
<td>156</td>
</tr>
</tbody>
</table>
After finding the corresponding modulus-weighted area moments of inertia, the ratio of bending moments of the coated and uncoated wires will be examined by assuming that an equal stress is applied to each, setting up the following equation:

\[ M_w \frac{r_w}{I_{\text{weighted} w}} = M_T \frac{r_T}{I_{\text{weighted} T}} \]

After setting up the equation to solve for \( M_T \), the following relation is determined:

\[ M_T = M_w \frac{r_w * I_{\text{weighted} T}}{r_T * I_{\text{weighted} w}} \]

If the magnitude of the coating’s elastic modulus is significantly smaller than that of the wire, the two modulus weighted area moments of inertia can be assumed to roughly equal. This assumption leads to a further simplification of the equation in case where the modulus of the wire is large, and the modulus of the coating is small:

\[ M_T \cong M_w \frac{r_w}{r_T} \]

In this case, the ratio of radii is the primary factor that influences the ratio of the bending moments is the radius of the coating and the radius of the wire. Using the full set of equations, relationships between the wire and the 3 coatings were established. Table 6 below shows the results when assuming a uniform coating radius is utilized.
### Table 6: Mechanical Calculations for Uniform Radii

<table>
<thead>
<tr>
<th></th>
<th>Uncoated</th>
<th>Chitosan</th>
<th>PCL</th>
<th>PLLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radius of Wire (in)</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>Radius of Wire (mm)</td>
<td>0.0762</td>
<td>0.0762</td>
<td>0.0762</td>
<td>0.0762</td>
</tr>
<tr>
<td>Radius of Coating (in)</td>
<td>0</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>Radius of Coating (mm)</td>
<td>0</td>
<td>0.1016</td>
<td>0.1016</td>
<td>0.1016</td>
</tr>
<tr>
<td>Estimated Modulus (MPa)</td>
<td>58000</td>
<td>6.3</td>
<td>156</td>
<td>1000</td>
</tr>
<tr>
<td>I of Wire (mm(^4))</td>
<td>2.64795E-05</td>
<td>2.64795E-05</td>
<td>2.65E-05</td>
<td>2.65E-05</td>
</tr>
<tr>
<td>I of Coating (mm(^4))</td>
<td>N/A</td>
<td>5.72088E-05</td>
<td>5.72E-05</td>
<td>5.72E-05</td>
</tr>
<tr>
<td>I Total (mm(^4))</td>
<td>2.64795E-05</td>
<td>8.36883E-05</td>
<td>8.37E-05</td>
<td>8.37E-05</td>
</tr>
<tr>
<td>Modulus Weighted I Uncoated (MPa * mm(^4))</td>
<td>1.535810952</td>
<td>1.535810952</td>
<td>1.535811</td>
<td>1.535811</td>
</tr>
<tr>
<td>Modulus Weighted I Coated (MPa * mm(^4))</td>
<td>N/A</td>
<td>1.536171368</td>
<td>1.544736</td>
<td>1.59302</td>
</tr>
<tr>
<td>Ratio of Bending Moments (Mc = x*Muc)</td>
<td>1</td>
<td>0.750176006</td>
<td>0.754358</td>
<td>0.777937</td>
</tr>
<tr>
<td>Ratio of Radii</td>
<td>1</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
</tr>
</tbody>
</table>

The above table shows that the coatings perform similarly when they are the same radius. The ratio of radii is best utilized as a numerical check, as it is the ratio of bending moments if the modulus-weighted area moment of inertia of the coating is negligible. The coating with the bending moment closest to the original is PLLA by about 2% over the other coatings examined. However, the coatings will not have uniform radii due to their coating thicknesses and ability to adhere to the stent wire. In Table 7, the coatings were examined using estimated radii from coating testing.
Table 7: Mechanical Calculation for Observed Radii

<table>
<thead>
<tr>
<th></th>
<th>Uncoated</th>
<th>Chitosan</th>
<th>PCL</th>
<th>PLLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radius of Wire (mm)</td>
<td>0.0614</td>
<td>0.0614</td>
<td>0.0614</td>
<td>0.0614</td>
</tr>
<tr>
<td>Radius of Coating (mm)</td>
<td>N/A</td>
<td>0.0677</td>
<td>0.0643</td>
<td>0.0907</td>
</tr>
<tr>
<td>Estimated Modulus (MPa)</td>
<td>58000</td>
<td>6.3</td>
<td>156</td>
<td>1000</td>
</tr>
<tr>
<td>I of Wire (mm^4)</td>
<td>1.114E-05</td>
<td>1.114E-05</td>
<td>1.114E-05</td>
<td>1.114E-05</td>
</tr>
<tr>
<td>I of Coating (mm^4)</td>
<td>N/A</td>
<td>5.369E-06</td>
<td>2.277E-07</td>
<td>4.202E-05</td>
</tr>
<tr>
<td>I Total (mm^4)</td>
<td>1.114E-05</td>
<td>1.651E-05</td>
<td>1.342E-05</td>
<td>5.316E-05</td>
</tr>
<tr>
<td>Modulus Weighted I Uncoated (MPa * mm^4)</td>
<td>0.646</td>
<td>0.646</td>
<td>0.646</td>
<td>0.646</td>
</tr>
<tr>
<td>Modulus Weighted I Coated (MPa * mm^4)</td>
<td>N/A</td>
<td>0.646</td>
<td>0.647</td>
<td>0.688</td>
</tr>
<tr>
<td>Ratio of Bending Moments (Mc = x*Mc)</td>
<td>1</td>
<td>0.906</td>
<td>0.955</td>
<td>0.721</td>
</tr>
<tr>
<td>Ratio of Radii</td>
<td>1</td>
<td>0.906</td>
<td>0.955</td>
<td>0.677</td>
</tr>
</tbody>
</table>

In this case, PCL is the closest to the original bending moment, due to its small coating radius. Chitosan performs as expected with the ratio of radii, and PLLA, while having the lowest ratio of bending moments, performs better than the ratio of radii. Knowing the ratios of the bending moments of the coated and uncoated wires allows for approximations of the applied forces to occur. Utilizing the derived form of the force applied to a beam, the following equations are known:

\[ M = \frac{\sigma I}{r} \]

\[ F = \frac{4M}{L} \]
Assuming the applied stress is 33 kPa, knowing the length is 2 in, and determining the radius and area moment of inertia above, the force required to reach 33 kPa in the uncoated wire can be determined to be $9.0315 \times 10^{-7}$ N. This is far below the Instron’s load cell and verifies the lack of observable data returned after testing. Knowing the ratios of the bending moments of the coated and uncoated wires, it is determined that it will take approximately 90% of the uncoated force for chitosan, 72% of the uncoated force for PLLA, and 96% of the uncoated force for PCL to reach the same stress. While these force values are still in the $10^{-7}$ range, it does alter the mechanical properties. With this in mind, the theoretical data should be weighed less than other testing components due to uncertainties in the properties of a coated stent, since these calculations considered individual wires.

5.4 Cytotoxicity Test Results

The MTT assay was done using 3T3 mouse fibroblast cells in DMEM basal media with penicillin-streptomycin, glutaMAX, and fetal bovine serum added to it. This standard curve was run as a proof of concept experiment to show that the number of cells seeded has a direct effect of the light absorbance recorded. This experiment was also done to identify the range for which this assay is valid. Based on the data gathered seeding anywhere from ten to fifty thousand cells after 48 hours of growth falls within the valid range for this assay. The data generated from this assay was then used to create a line of best fit which was later used to find the number of living cells detected after they were exposed to various leachates as shown in Figure 25.

![Figure 25: Creation of Leachates for MTT Assay](image)

This standard curve is displayed below in Figure 26.
The results from the MTT assay are shown below in Figure 27. These results show that little to no cell death occurred after seeding the cells at about 40,000 cells per well originally. Some of the variation can also be attributed to human error when seeding the cells originally. The lack of cytotoxicity is also evidenced by the lack of increased cell death in the 14-day leachates.

5.5 Adhesion Testing Results

The uncoated and coated wires underwent cell adhesion testing to determine if the addition of a coating increases a stent’s ability to adhere to tissue. To perform this type of testing, a non-adhesive well was utilized, preventing the cells from sticking to anything but the selected wire sample. Additionally, said well was designed with a V-shaped notch to allow gravity to increase the chances of the cells settling directly on to the wire, as opposed to evenly spread around the well, lacking contact with the wire sample [55].
Agar was selected as the material for these wells, due to it being non-adherent to cells. In the initial well design, the triangular prism-shaped strips of PDMS were utilized to leave a v-shaped impression on the agar. This PDMS was made utilizing a 10:1 ratio of Sylgard base and curing agent, which was thoroughly mixed, poured into a petri dish and then degassed for approximately hour. After degassing, the PDMS was cured in an oven for an hour at 60°C, removed and allowed to cool. Following this, the PDMS was cut into triangular prism-like strips approximately three fourths of the length of a 6-well plate well with a razor blade. Alongside these PDMS strips, a roughly 2% agar solution was made using 500mL of de-ionized water and 11g of agar. This solution was placed in the autoclave in a glass bottle with a loose cap (to prevent the pressure from shattering the bottle) alongside an autoclave pouch containing the individual PDMS strips along with forceps and a spatula to assist with the placement of the strips into 6-well plates.

After laying the strips along the bottom of the wells, 10 mL of warm hot agar was transferred into each well using a pipette controller. The agar was cooled in a sample refrigerator and removed when the agar was a solid gel. A spatula was utilized to flip the well over and remove the PDMS strip, completing the wells. They were stored in the 6-well plates in the refrigerator while being inverted to prevent any unwanted bacterial growth from occurring. Figure 28 displays the wells as they were cooled into a gel in the refrigerator and Figure 29 displays the results of the well molding.
The second well design was nearly identical to the original, but it utilized 3D printed polyethylene terephthalate glycol-modified (PETG) mold to accurately replicate PDMS strip sizes in every sample. The mold was printed on a Wanhao i3 3D printer with micro-swiss all metal
hotend, and used MakerGeeks 1.75mm HD Blue Glass PETG filament. The advantage of using a mold is that it ensures that each strip is of uniform length and depth, preventing any human error from occurring while cutting PDMS to approximate sizes. This 3D printed mold is displayed below in Figure 30 below.

![3D Printed PDMS Mold, Designed by Washburn](image)

PDMS was poured into the mold until about 1-2mm was left unfilled per mold compartment. This was degassed for 30 minutes, then cured in the oven for 1 hour. The resulting PDMS gels were autoclaved and underwent the same process as the individually cut PDMS notches.

Media was added to each used well of a six well plate along with the agar V-shaped mold containing a wire sample so that both the mold and wire could equilibrate with the media. This allowed for adhesion testing to occur following cell seeding. Figure 31 displays the wells following loading with media.
After seeding cells onto the wires using these molds Hoechst dye was added to make the cells visible. There were issues imaging the wire samples. The thick layer of agar scattered the light from the microscope which made it nearly impossible to properly image the samples. The wire samples were removed from the molds, but few cells remained after being pried up out of the wells and placed onto glass coverslips. It was also found that the chitosan polymer took up the Hoechst dye that was used to stain the cells which made it impossible to tell the difference between any possibly adherent cells and the polymer coating as seen in Figure 32.
A second round of testing was done without the agar molds to improve imaging and with GFP cells to make the cells fluoresce green. However, similar issues occurred. While cell accumulation around each of the wires is visible each of the polymer coating shown in Figure 33 it is difficult to tell if these cells are truly adherent to the wires or just accumulating around them. It was also noted that even when the cells were spun out of the Hoechst dye before they were seeded onto the wires the chitosan sample still fluoresced which indicates that it is auto fluorescent.
5.6 Cost Analysis

The cost to apply each coating is based on the amount of each polymer required per complete dip coating, approximated utilizing known prices per quantity of polymer. This, in addition to any solvent cost, will determine which coating is the least expensive to utilize, and will be ranked accordingly. These costs can be observed numerically in Table 8 and comparatively in Figure 34 below.

Table 8: Production Cost of Coatings Per mL

<table>
<thead>
<tr>
<th></th>
<th>Chitosan</th>
<th>PCL</th>
<th>PLLA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cost per mL of Polymer Solution</strong></td>
<td>$0.03</td>
<td>$0.43</td>
<td>$3.33</td>
</tr>
</tbody>
</table>
In summary, chitosan is the least expensive coating to produce per mL, while PLLA is the most expensive to produce per mL of solution.

Figure 34: Bar Graph of Production Cost per mL of Polymer Solution
6.0 Final Design and Validation

6.1 Methodology Summary

6.1.1 Coating Integrity Methods

To coat a nitinol wire sample in a chosen polymer, the polymer must first be dissolved in a solvent. Some polymers require specific solvents, but acetone and chloroform are commonly utilized. The mass of polymer used depends on the desired mass% for the final solution. For example, a 10% w/v solution with a volume of 100 mL would use 10g of solid polymer and 90 mL of solution. Varying the mass% will change the final properties of the solution, with the viscosity of the solution increasing at higher % w/v values. The solubility may be different for each polymer. For the polymers used in this paper, the solid PCL and PLLA could be dropped into their solvents, left over night in a 40C incubator, and they would be dissolved in the morning. PCL also needed to be shaken thoroughly at the end to ensure even mixing. Dissolving chitosan was more difficult. The solid chitosan dissolves into a very viscous solution, so any undissolved polymer becomes more difficult to dissolve as times goes on. To solve this, the chitosan needed to be stirred while in the Acetic Acid, after which the solution would be ready in about two hours. Once the polymer solution was prepared, one must also prepare any additional solutions needed. An example of this would be chitosan from the experiments represented in this paper. Chitosan’s solvent was 5% acetic acid, which does not readily evaporate as acetone and chloroform do. So, the solvent needed to be reacted out of the solution once it has been applied to the sample. For chitosan, this second solution, noted as the fixing solution, was a mixture of ethanol and ammonia.

Once the solutions had been prepared, the wires were dipped into the solution. In the experiment from this paper, the wire samples were dipped into the polymer solutions for two minutes. Additionally, the chitosan samples were then dipped into the fixing solution for five minutes. Following dipping, the samples were placed into prepared vials, using small binder clips to keep them suspended while they dry. The vials that were used tended to be unstable, so a tube and base complex was designed and 3D printed to keep the vials from falling while they sat in the incubator at 40C. The drying process was complete in about 24 hours. When the samples were completely dry, they needed to be dipped one more time to help removed any excess solvent. For PCL and PLLA, this dip was in deionized water. For chitosan, the samples needed to have one more dip in their fixing solution.
As soon as the samples were dry, they were ready for imaging. The wire samples were put into a petri dish for the imaging process. The samples tended to get statically stuck to the dishes, so the binder clips were left on the samples for easy removal. The samples were imaged at 20x magnification, and if the coating was not even the samples were searched for any evidence of a coating before the image was recorded. Once the samples were imaged, they were returned to their vials, released from their binder clips, and capped off so that the samples would be ready for the next experiment they would be used in.

6.1.2 Application Concerns Methods

The application concerns become more of an analysis of properties of the polymers and their solvents. The focus was more on the analysis of the polymers ability to coat the wire samples. For chitosan, it could a coating averaging around 6 microns thick, but the coating was overall very uneven. The polymer tended to bead on the wire rather than maintain an even coating. For PCL, the polymer could create an even but thin coating. For PLLA, the coating tended to be very even and thick, instead. The importance of the thickness of the coating depends on the purpose of the coating. As the wire samples were representing a canine tracheal stent, where a thick coating could impair the stents ability to shrink and expand, a thin coating would be preferred.

For the solvents, the application concerns came mostly down to safety. Exposure to acetone and acetic acid is common, in the form of nail polish remover and vinegar respectively, so they do not pose a hazard for human operators. However, chloroform has been tested and found to be toxic and carcinogenic with long term exposure. This could be a danger to those applying the coating regularly if proper safety conditions are not met.

6.1.3 Mechanical Integrity Methods

As examined in Chapter 5, the normal 3-point bending methods for solid samples (utilizing the Instron 5544) was not applicable to the stent wire samples due to their size and ability to freely bend. This resulted in the need to derive a theoretical calculation to examine the overall impact that adding a coating has on the ability of the wire to resist forces applied to it. This was done by utilizing both the general bending moment calculations for an uncoated and coated wire sample, setting all variables equal to an arbitrary stress value, along with implementing the modulus-weighted area moment of inertia to better understand the magnitude of the contribution that the coating has on the mechanical properties of the individual wires.
Adding a coating to the stent wires may result in a change in the mechanical integrity of the wire itself. In general, simple 3-point bending is utilized to relate changes to force and displacement based on the coating added to the wire. However, due to the small size of the individual wires examined, the mechanical testing device, an Instron 5544, was unable to record the force applied to the wire because of the difference in magnitude between the force and the load cell resolution for measuring forces. Theoretical calculations are utilized to estimate the mechanical effects adding a coating has for a stent wire.

The bare wire is assumed a solid cylinder of radius $r_w$, while the coating is assumed to be a hollow cylinder with an outer radius $r_T$ and an outer radius or $r_w$, where $r_w$ is the radius of bare wire and $r_T$ is the radius of the wire and coating together. A visual representation of the composite consisting of the wire and coating can be seen below in Figure 35.

![Figure 35: Wire and Coating Composite](image)

The modulus-weighted area moments of inertia were derived by multiplying the composite components, the wire and the coating, by the appropriate elastic modulus. The coated wire required the summation of the two components, while the uncoated wire utilized the wire modulus only. After deriving the modulus-weighted area moments of inertia, the general bending moment equation is set up for both the uncoated and coated wires. Following this, the applied stresses are set equal, allowing for the following equivalence statement to be derived:

$$M_T \frac{r_T}{I_{\text{weighted}_T}} = M_w \frac{r_w}{I_{\text{weighted}_w}}$$

$$\frac{M_T}{M_w} = \frac{r_w * I_{\text{weighted}_T}}{r_T * I_{\text{weighted}_w}}$$
This ratio of total (wire and coating) bending moment and bare wire bending moment determines difference in bending moments between the two samples for the same applied pressure. This ratio correlates to the force applied to either wire sample, where if the ratio is less than 1, then it will take less force for the coated wire to reach the same stress as the uncoated wire, while if the ratio is greater than 1, then it will take greater forces on the coated wire sample to reach an equivalent stress as the uncoated wire. This is essentially determined by the following ratio:

\[
F_T = \frac{M_T}{M_W} F_W
\]

With this relation established, the mechanical effects of applying a coating to a wire can be examined theoretically. While this method allows for simple verification, utilizing a mechanical testing device with a sensitive load cell or larger wire samples is recommended where possible.

### 6.1.4 Cytotoxicity Methods

The possible cytotoxic properties of each of the materials used was assessed by using an MTT assay. First a standard curve of known values of live cells was evaluated to find a line of best fit that could then be compared to unknown values in order to find their cell viability. The experimental cells were made by first soaking each type of wire sample in media for either 7 or 14 days. The leached media was then removed and used to culture the experimental cells. Overall this test showed that none of the materials tested were cytotoxic.

### 6.1.5 Cell Adhesion Methods

The tissue adhesion properties of each of the materials was tested by using cells that were cultured with wire samples. However, after running into problems with imaging the wire samples literature was referred to in order to figure out the adhesive properties of each polymer [29].

### 6.1.6 Cost Effectiveness Methods

The cost effectiveness of each polymer was found by determining the cost of producing an mL of the polymer solution. To perform this calculation, the cost of the polymers per gram and the solution concentrations, in grams of polymer per mL of solution, were found. These values were then multiplied together to calculate the cost per mL of solution. For the polymer candidates, the cost per mL was found to be $0.03 for 2% Chitosan, $0.43 for 5% PCL, and $3.33 for 5% PLLA.
6.2 Summary of Data Analysis

Once the testing methods had been carried out, the data could be analyzed, and the results compared. For the coating consistency, the results showed that chitosan produced a coating of about 6.5 microns thick, PCL produced a coating about 3 microns thick, and PLLA produced a coating about 30 microns thick. These values were an average, and each of them had a standard deviation that was larger than the average value. PCL was considered to have a coating that was too thin, and it would likely not withstand the stresses of standard canine tracheal motion. PLLA had the opposite problem, where the coating was decided to be too thick, which would impact the ability of the stent to deploy. Thus, the chitosan coating was found to be the preferred coating.

For manufacturing concerns, it was decided that PCL was lacking due to its difficulty of producing a coating. Likewise, PLLA was also found lacking due to too great of an ability to form a coating. Also, PLLA requires the use of a toxic solvent, chloroform, which is carcinogenic after long exposure. This would pose a danger to anyone operating equipment to coat the stents. Thus, we were left with chitosan, as it’s solvents and other necessary chemicals are all readily available in a department store.

Analysis of the mechanical integrity found that both chitosan and PCL had very little effect on the mechanical strength of the nitinol wire. The applied force needed to generate a certain stress in the chitosan and PCL coated wires was only about 90% of the force that would needed to generate the same stress in an uncoated wire. PLLA, on the other hand, caused a decrease in the required force to produce an equivalent stress by about 30% of the force required for an uncoated nitinol wire.

Cytotoxicity testing found that all of the polymer coatings were as non-cytotoxic as an uncoated nitinol wire. The comparison between the polymers found no difference, and the comparison within each polymer between the samples that had been leached for 7 days and those that had been leached for 14 days found no difference. That is, there were 40,000 cells seeded in the leachates from the polymer cells and the bare wires, and there was no significant cell death caused by any of the leachates.

As the cell adhesion testing ran into a large compliment of errors, the data was decided to be unusable in the state it was. As such, with the time available to the team at that point, it was
information from published materials was used to provide these results. From these published materials, it was found that chitosan proved to be mucoadhesive, which would be beneficial in the canine trachea. PCL and PLLA, however, both showed results that their application as coatings decreased the overall cell adhesion. As such, chitosan was decided to be the preferred material.

Finally, an analysis of the costs associated with the production of the polymer solutions was done. From this analysis, it could be seen that chitosan was the most affordable of the three polymers, with PCL coming in a close second place and PLLA trailing far behind in third.

6.3 Final Candidate Selection

Following the completion of all tests, the results were examined using the weighted design matrix. The candidate coatings were compared against one another and against the bare nitinol wire (gold standard) with the following rating values: 0 – meets gold standard; +1 – exceeds gold standard; -1 – falls below gold standard. The weight of each test was determined based on the importance of each feature to the overall design. These weights were then multiplied against the score values for each candidate in each test, and the highest overall score determined the selection of the final candidate coating. Table 9 below illustrates the score calculations.

<table>
<thead>
<tr>
<th></th>
<th>Bare Wire</th>
<th>Chitosan</th>
<th>PCL</th>
<th>PLLA</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coating Consistency</td>
<td>0</td>
<td>+1</td>
<td>-1</td>
<td>-1</td>
<td>2.5</td>
</tr>
<tr>
<td>Manufacturing Concerns</td>
<td>0</td>
<td>0</td>
<td>+1</td>
<td>-1</td>
<td>0.5</td>
</tr>
<tr>
<td>Mechanical Integrity</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-1</td>
<td>1.5</td>
</tr>
<tr>
<td>Non-Cytotoxic</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.5</td>
</tr>
<tr>
<td>Cell Adhesion</td>
<td>0</td>
<td>+1</td>
<td>-1</td>
<td>-1</td>
<td>3.0</td>
</tr>
<tr>
<td>Cost</td>
<td>0</td>
<td>+1</td>
<td>-1</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>+6.5</td>
<td>-6.0</td>
<td>-8.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>

As can be seen, the chitosan coated wire performs better than the PCL and PLLA coated wires. The chitosan dip coated stent was chosen to be the final proposed candidate for this project.
This candidate produced approximately a 6.5-micron coating. Additionally, manufacturing the coated stent with this polymer presents minimal difficulties due to its non-toxic solvent, acetic acid. However, the coatings produced were uneven in some cases, but utilizing a modified dip coating procedure or a mechanized process could help to ensure that future coatings are even. The coating increased the mechanical forces applied to the stent by approximately 10%, which corresponds to the ratio of the uncoated and coated wire radii. This force difference is expected as the total radius increases, and it performs better than PLLA’s ~28% difference. When looking at cytotoxicity, chitosan performed no better than the other samples. However, all the samples showed no evidence of any cytotoxicity. After examining the per mL cost of each coating solution, chitosan was determined to be the least expensive, presenting the best value for a veterinarian or manufacturer looking to apply the coating as a post-production modification.

The final candidate was selected by weighing what qualities were the most important for a successful product and then comparing each of the possibilities to a bare nitinol stent which is currently the gold standard. From this comparison it was clear that a chitosan coated nitinol wire was by far the best choice. A chitosan coated wire is non-toxic, does not hinder the mechanical properties of the nitinol wire, does not require the use of toxic chemicals during its fabrication, is tissue adhesive, and is the most affordable option that was assessed. ISO standards were considered when deciding what properties needed to be considered to ensure that the final candidate choice was biocompatible. The ISO standards that were focused on were for cytotoxicity, implantation, and degradation. The MTT assay was used to address any cytotoxic concerns. Adhesion testing and literature was used to ensure a positive implantation with reduced amounts of inflammation and scar tissue build up. Finally, the degradation properties were assessed using mechanical equations. In order to market this device, the adhesion testing would have to be improved so that literature did not have to be relied upon. Manufacturing considerations would also have to be taken like packaging the coated stent in sterile packaging.

6.4 Impact of Final Candidate

When creating a new method or apparatus, its impacts on the world at large must be theorized before public release. These impacts can range widely, from economic and environmental all the way down to manufacturability and sustainability. To ensure that the variation is making positive impacts, or may potentially in the future, all influenced aspects need
to be considered during the production and experimentation process. These potential impacts influenced the development of our final candidate coating.

6.4.1 Economic

Canine medicine is expensive. As such, minimizing the cost of medical treatment allows for dog owners to be able to consider better solutions to medical issues experienced by their dogs. The application of this coating may reduce the chances of stent failure due to fracture or immune response. While the coating will result in an increase to the stent price, the need for emergency surgery and stent replacement will be reduced upon improving the coating to market level, resulting in an overall decrease in the price sustained tracheal collapse treatment. This will in turn allow for the procedure to be more economically accessible to dog owners due to increased success rates.

6.4.2 Environmental

The new canine tracheal stent coating should enhance the lifespan of stents, so there will need to be less disposal of broken stents. The polymer used for the final coating design, chitosan, is derived from crab shells. In the case where the coating becomes commonly utilized in tracheal collapse treatment and for other purposes, then overfishing of crabs and other shellfish to harvest chitosan could become a potential problem. This may also allow for a reduction of waste if these shells are obtained from sources that primarily utilize the meat of these crustaceans. The only significant environmental impact could come from the disposal of the solvent and chemicals used during the coating process. While there are varying levels of toxicity among them, all of the solvents utilized would be considered hazardous waste. Inappropriate disposal of said chemicals, such as unregulated dumping into water sources, could lead to major ecological impacts and organism death.

6.4.3 Societal

Dogs are popular companion animals globally. It can be detrimental to the mental state of dog owners to witness their dogs suffer from or die of medical complications. By enhancing the treatment of a disease that reduces dogs’ quality of life, these dogs can sustain their health and live longer. A treatment such as this will likely be viewed favorably by the general populace.
6.4.4 Political

The new chitosan coating for canine tracheal stents falls within the current regulation for medical stents, so there should not be political ramifications from the coating’s release.

6.4.5 Ethical

Canine tracheal collapse results in the reduction of the quality of life of dogs. Canine tracheal stents exist to reduce the effects of this condition, and in turn improve the dog’s quality of life. However, the occurrence of stent failure results in increased distress, emergency surgeries to retrieve and replace the faulty stent, and an additional recovery period following the ordeal. With an improved stent coating, the likelihood of failure occurring decreases, allowing for less suffering and distress to occur following surgery.

6.4.6 Health and Safety

Dogs tend to contract canine tracheal collapse around 7 years-of-age on average. As many of the dogs that can suffer from this condition have lifespans up to 14 years, tracheal collapse can reduce their lifespans significantly. The failure of a stent would additionally cause serious harm to the dogs affected. As such, the introduction of a new coating for canine tracheal stents that can extend the lifespan of these stents, reducing the chances of failure and allowing for extended treatment of tracheal collapse, would increase the health of the dogs affected by canine tracheal collapse overall.

6.4.7 Manufacturability

This new coating should be easy to recreate and apply to current stents in a manufacturing setting. This is supported by its production using standard laboratory equipment, suggesting that mass production would not pose issues. In a manufacturing setting, the polymer solutions would be able to be produced in mass and machines would be able to dip more stents at a faster rate than a standard laboratory technician can. The simple process would require very little additional work to move the production of the coating to a manufacturing setting. Additionally, manufacturing machinery could ensure consistent quality of coatings with precision not attainable without machine assistance.
6.4.8 Sustainability

This new coating requires minimal energy input to produce. A stirring mechanism is required to prepare the chitosan solution, and an incubator or oven is required to dry the coatings. Aside from these energy uses; the new coating has minimal impact on renewable energy and energy consumption.
7.0 Discussion

7.1 Analysis of Results

7.1.1 Coating Integrity

The primary mechanical processing aspect of this project is the application of the chitosan coating to the nitinol wire. If a coating does not stick, or does not coat evenly, then it may not provide the beneficial properties like a normal coating would. As such, there are many aspects of this process that can vary by individual sample. These aspects are concentration, preparation, and speed of removal from the solution.

Concentration is the most basic aspect of the coating process. Most polymer solutions are made using a percent weight per volume (\% w/v) value, which represents the percentage of the total mass of polymer in the solution. In the development process of the chitosan coating, the mass percent used was 2\% w/v, which was a value obtained during the literature review [49]. This produced a very viscous solution. At a later point in the process, an additional 5\% w/v solution was accidentally produced. This resulted in a solution that was almost completely solid. This large difference in results between two \% w/v values is likely due to the nature of chitosan as a polymer itself. Chitosan is a biological polymer derived from the chitin in crab shells. Crab shells are the crabs’ natural defense against predators, and the strength of the chitin that makes up the shells is integral to this. Chitosan retains some of chitin’s properties, most notably its random amorphous form in its polymerized state. This amorphous quality allows the chitosan solution to have higher viscosity at lower \% w/v of solid polymer.

The polymer preparation method could have caused this viscosity change. Chitosan requires 5\% acetic acid to dissolve and doesn’t react to acetone or chloroform like PCL and PLLA. However, 5\% acetic acid is a significantly weaker solvent than either acetone or chloroform. There are other aspects of preparation, though. Unlike PCL and PLLA, chitosan required stirring for the entirety of its dissolution, or the solution would become too solid for it to be reliably used to coat the samples. The duration of mixing, which was minimally 2 hours, could have influenced viscosity differences. As in, if the solution had been mixed longer it may have resulted in a less viscous solution. One additional factor that was discovered during the course of experimentation was temperature exposure. By default, the chitosan was on a heated stir plate during preparation.
This did not result in even heating. A sample vial that had been left in the incubator, in case it was needed to produce more samples, ended up being kept at 40°C for several days. In contrast to the chitosan that was used during the sample production, this vial was quite liquid and not viscous at all.

All the chitosan samples appeared as though they had beads of chitosan down their length, and that results in the wavy uneven quality that can be seen in the sample images. The speed that the samples were removed from their solution dips results in different physical properties for the coatings. If the samples are removed quickly, thicker coatings are produced. Removing the samples from their polymer slowly allows the polymer solution to slide down the sample and back into the mixture. Thus, removing the samples faster allows less to drip and creates a thicker coating. The samples will likely have been removed at varying speeds due to human variability. Varying the speed during a single removal could result in an uneven coating. Additionally, bumping the sample into the sides of the vial, or jolting the sample as it is moved can alter the surface consistency. These variabilities could have been removed using an automated process.

### 7.1.2 Manufacturing Concerns

While the observable physical properties of the coatings themselves were closely analyzed and monitored during the testing, other aspects for manufacturing the coatings were also considered. Most notably, the safety of producing the polymer solution. Out of the three polymers that were tested, chitosan, PCL, and PLLA, the chosen polymer, chitosan, posed the least danger in its components for production. While PLLA required chloroform and PCL required acetone, both of which evaporate easily and produce toxic fumes, chitosan required only 5% acetic acid for its solvent, which is essentially vinegar. Aside from its weak solvent, chitosan required other fluids to fix it to the nitinol samples, ethanol and ammonia. Ethanol is the primary alcohol found in alcoholic beverages, and ammonia is a commonly used household cleaner. These chemicals are all safe enough to be readily purchasable for regular use at any department store.

### 7.1.3 Mechanical

Due to scale limitations with the available mechanical testing system (the Instron 5544’s 20N load cell), theoretical 3-point bending calculations were utilized. As such, the results of these calculations presented approximations of the applied force changes on the uncoated and coated wire samples. This was done by utilizing modulus-weighted area moments of inertia to examine
the contributions of both the wire and the different coatings. When comparing the bending moments of the uncoated and coated wires, it was apparent that the largest factor the resulted in a difference in the moments was the radius of the examined coating, since the moduli of all coatings were significantly smaller, ranging from 50 times smaller to nearly 10,000 times smaller than that of nitinol. As such, in the case of chitosan and PCL, the contribution of the elastic modulus was minimal, while the PLLA coated wire’s bending moment ratio to the uncoated wire was reduced by an additional 5% when compared to the ratio of the radii of this coated wire and its uncoated counterpart. This implies that the coating’s mechanical properties do not impact the bending moment of the wire as strongly as the increased radius. As such, the optimal thickness of the coating should be considered to prevent the mechanical integrity of the nitinol stent wires from lowering, which may lead to premature stent failure.

7.1.4 Cytotoxicity

Whether or not each of the materials tested were cytotoxic was a pass/fail test for if they could possibly be used as a long term implanted device. While this test was critically important all of the materials that were tested passed so it did not affect the overall coating material selection process.

7.1.5 Cell Adhesion

Cell and tissue adhesion is critical to the reduction of stent migration. By adhering the stent to the walls of the trachea it will be unable to move around and cause irritation and inflammation. The adhesion testing results used did not have clear results due to not being able to definitively tell whether cells had actually adhered to the wire samples or just accumulated around them, which resulted in literature being referred to instead of experimental data. It was clear from the literature that chitosan was the only polymer tested that had mucoadhesive properties [29].

7.1.6 Cost Effective

While cost may have been the last aspect of the chitosan coating that was evaluated, it is no less important. Originally, it was decided that the cost of the coating needed to be less than $400 to keep the coated stent affordable. That goal was fulfilled due to the low cost of the chitosan dip coating. The cost to create on milliliter (mL) of the chitosan solution amounts to approximately $0.03, or $30/L. To put this in perspective, the samples produced in these tests were two-inch-long
samples of nitinol wire. Using a liter of the chitosan polymer solution, almost 550,000 coated samples could be created, under the assumption that they all produced an even coating of identical thicknesses.

7.1.7 Summary

Overall, chitosan generally performed better than the competing polymers, PCL and PLLA, against the design objectives. While the average coatings of chitosan on the wire samples were uneven in thickness, they were at least consistently present. On PCL, it was uncertain if there was a successful coating every time. The chitosan coatings were also not thick enough to have much of an effect on how much force is needed to produce a stress in the wires. PLLA, on the other hand, produced coatings that were thick enough to decrease the required force by 30%.

All the polymers tested passed the cytotoxicity testing. The uncoated wire left approximately the same number of cells alive as the number of cells exposed, and the polymer coated samples performed the same. The only way for one of the polymers to perform better would have been for it to increase the overall cell growth.

The adhesion testing was never successfully completed. There were always some issues, ranging from staining issues to imaging issues to cell issues. Looking directly at chitosan, it potentially caused some of those errors. Chitosan is a biological polymer, so it can apparently take up some of the nucleic acid dyes used on cells. Moreover, even when there was careful preparation to ensure that no nucleic dye made it to the chitosan, chitosan would prove to have some innate autofluorescence. Taking this new information, it is possible that the original fluorescence of the chitosan was not due to the absorption of dye, but the inherent physical properties of the chitosan. This would cause issues until there was no longer any time to test. Going back to the research materials, chitosan was decided to have better adhesion based upon it’s mucoadhesive properties [30].

Finally, chitosan came out on top of the cost analysis. When cost was calculated on a volume of solution basis, chitosan’s cost was found to be $0.03/mL. PCL was found to be an order of magnitude above that value at $0.43/mL, and PLLA was an additional order of magnitude greater at $3.33/mL.
7.2 Limitations

The primary limitation of the chitosan coatings is attributed to the inconsistency of the coating. Through all the coating samples, the chitosan never produced an even coating. When it was removed from the polymer solution, the chitosan would always bead along the wire. Theoretically, chitosan should have produced nice coatings due to its viscous nature when in a solution with acetic acid. However, this was never evidenced.

Relating back to the literature, chitosan’s irregularity can be noted once more. Its overall degradation is neither bulk nor surface, but somewhere in between. This can make it very difficult to predict the degradation period, and also makes chitosan’s degradation period irregular. While this point of chitosan’s nature was not directly studied in these experiments, it would a have been one of the major aspects that would have needed to be studied once the polymer was chosen as the primary coating. It wouldn’t do any good for the coating to spontaneously break down shortly after the stent it is on is implanted. That would negate the whole purpose of the coating.
8.0 Conclusions and Recommendations

8.1 Conclusions

The primary goal of this project was to design a product or process to improve existing stents and to promote the quality and longevity of canine tracheal collapse treatment. The objectives for the development of said product that were chosen to meet this goal were to reduce stent migration, maintain the trachea opening, be compatible with current manufacturing methods, and be low cost. Once it was decided that the product created in this project would be a coating for existing stents, the testing methodology was created. This methodology was a series of tests meant to validate that objectives were met and validate that the final design met the overall goal of the project.

The methodology followed in this project was to test the overall consistency of the tested coatings, analyze the manufacturing concerns of the coatings, test and identify the effects of the coatings on the mechanical properties of the nitinol wire, test the cytotoxicity of the coatings, test the overall ability of cells to adhere to the coating, and analyze the price of the coatings. The three polymers that were tested were chitosan, PCL, and PLLA.

When analyzed for coating consistency, it was determined that chitosan consistently produced quality coatings while PCL and PLLA did not. This was because, while chitosan produced uneven coatings, the thin or thick coatings of PCL and PLLA, respectively, were detrimental. The miniscule PCL coatings and the thick PLLA coatings presented manufacturing concerns. Chitosan, however, did not present any of these concerns. Looking at the mechanical integrity of the samples, the only polymer that increased the stress applied to the wire was PLLA. It weakened the overall samples by approximately 30%. Chitosan and PCL both decreased the strength of the samples as well, but at most by 10%, which was decided to be negligible. The cytotoxicity testing found that all polymer coatings were comparable to the current bare nitinol in that they did not increase or decrease the viability of the tested cells. As the adhesion testing failed in general, looking at the literature was required to conclude this test. Based on what was found, it was decided that chitosan passed while PCL and PLLA failed. This was because chitosan has been shown to exhibit mucoadhesive properties, while both PCL and PLLA showed records of allowing
more freedom of movement [30]. Finally, looking at cost, chitosan had the best cost at $0.03 per mL, PLLA has the worst cost at $3.33 per mL, and PCL was neutral with a cost of $0.43 per mL.

Going over the results of the testing, chitosan was chosen as the final candidate for the polymer stent coating. While chitosan did perform neutrally in several of the tests, it also was the only tested polymer to perform positively in any of the tests. Both PCL and PLLA only showed neutral or negative results, with PLLA having more negative results than PCL. In summary, this project finds that chitosan fulfilled the project objectives better than the bare wire, PCL, and PLLA. This coating would increase the overall longevity of the stents by decreasing the micromotion of the stent in the trachea, which should decrease the long term swelling and stenosis that tends to develop around canine tracheal stents and decrease their lifespans.

8.2 Future Work

After reaching the limit of the time that the team had available to work on this project, there were still several aspects of the project that were left incomplete. Additionally, new aspects were determined to be important for further developing this coating. Most notable and obvious of these was the need to continue with adhesion testing. There was not a successful conclusion reached from the adhesion tests that were performed, as the tests had all failed in some capacity. The major reason that these tests failed is likely due to chitosan has the property of autofluorescence. As such, any future adhesion testing would need to account for this. From the images that were taken, it appeared that the chitosan fluoresced blue. So, to fix this, it might be beneficial to utilize red and green dyes on the cells rather than the green and blue dyes. Other possible methods to test the adhesion that could be used are using a nitinol sheet instead of a wire, giving the cells a larger surface area to attach to, or using a bundle of wires.

Throughout the testing for this project, a solution of 2% w/v chitosan and 5% acetic acid was used. However, it was not measured properly at one point during the process, resulting in the concentration being closer to 5% w/v chitosan. The resulting solution was significantly more viscous, to the point of nearly being solid. As such, a further point of research would be to vary the concentrations of chitosan and acetic acid in the samples to examine what effects that would have on the final coatings. Due to monetary constraints, it was decided that this testing was beyond the project scope.
For the imaging of the samples, a microscope at 20x power was used. This only produced images of a resolution that is less than 1 microns. Originally, it was intended that Scanning Electron Microscopy (SEM) would be used on the chosen final products to characterize them in even more detail, as it has a resolution of 10 nanometers and would have shown even the thinnest of the coatings we produced. However, due to time constraints, this never occurred. Further testing should involve the use of SEM to verify coating thickness with higher accuracy than the utilized imaging methods. Additionally, SEM can validate coating distribution and topography.

A point that it is necessary to note is that only single wires were coated during these tests. While the wires were being used as stand-ins for actual stents, they lacked some of the mechanical properties that result from the stent being made from a wire mesh. To this end, it would be important to perform the tests again on a whole stent that had been coated. The mechanical 3-point bending tests could be validated on the whole stent using physical 3-point bending. As the actual tests were inconclusive and theoretical calculation were used instead, it is vital that the actual mechanical properties of the coated stent be tested. The theoretical calculation only represents the mechanical properties of a single wire, and the stent could potentially react differently when coated.

Upon successful refinement and validation of the coating method, it would be important to perform *in vivo* testing on the coated stents. All the experiments performed in this project were designed to be *in vitro* representations of *in vivo* conditions, but there were limitations in some replications of these properties. For example, the adhesion testing would benefit from being performed in a mucosal environment, so the mucoadhesive properties of the chitosan could have been verified. However, the reproduction or collection of mucosal tissue was beyond the capabilities of the team. *In vivo* testing would provide the correct environment to confirm the mucoadhesive properties of chitosan, as well as the stent’s behavior when it is deployed in a functional, living system.
Appendix A: Experimental Methods

Coating Methods:

**Chitosan Dip Coating Method**
Dissolve the Chitosan in 5% Acetic Acid [49]
2% w/v
40°C with stirring for 1.5 hr
Dip the nitinol wire into the Chitosan solution for 2 min
Move the dipped wire to the fixing solution for 5 min
The fixing solution should be 2 parts ammonia to 1 part ethanol [56]
Allow dipped wire to dry for 24 hr, at 40°C
Wash the dipped wire with the fixing solution
Just to make sure no acid remains
Allow the dipped wire to dry at 40°C

**PLLA Dip Coating Method**
Dissolve the PLLA in chloroform [47]
5-10% w/v (5% w/v for experiment)
Stirring at room temp
Dip the nitinol wire into the solution for 2 min
Remove wire and allow to dry for 24 hr @ 40°C
Rinse with DI water
Allow to dry at 40°C

**PCL Dip Coating Method**
Dissolve the PCL in acetone [48]
1-5% w/v (5% w/v for experiment)
Stirring at room temp for up to 6 hours
Dip the nitinol wire in the solution for 2 min
Remove wire and allow to dry for 24 hr @ 40°C
Rinse with DI water
Allow to dry at 40°C

**PDMS Mold Protocol**
- Weigh 100 grams of silicon polymer base and 10 grams of silicon polymer curing agent per batch
- Mix both together thoroughly and poured into PETG mold with v-shaped notches, until about filled 1mm from top of mold (this allows for the PDMS to become a negative of this, allowing the agar wells to obtain v-shaped notches) [55]
  - Printed on a Wanhao i3 3D printer with micro-swiss all metal hotend
  - Printed using MakerGeeks 1.75mm HD Blue Glass PETG filament
- Degas in degassing chamber for 30 minutes, depressurizing the chamber as bubbles reach the surface of the PDMS
- PDMS cured in mold in oven at 60°C for 1 hour
- PDMS gel negatives removed from the mold using forceps and a spatula
- Placed in autoclave pouch with necessary instruments place in 6-well plates and to flip solidified agar
- Autoclaved on dry cycle

**Agar Well Protocol**
- Autoclaved 500 mL of 2% agar dissolved in deionized water solution on liquid cycle
- PDMS negatives placed in 6-well plates with ridges upward
- Warm PDMS poured into 6-well plate using pipette controller
  - Approximately 8 mL per well
- Taped plates closed, allowed to cool refrigerator
- Following cooling, solidified agar wells are flipped over in plate, PDMS is removed and disposed of, agar wells plated notch up
- Taped plates closed, stored inverted in refrigerator until time of use

**MTT Assay**

**Materials**

- Reagent
- Tissue culture 96 well plate
- 100 µL pipet

**Protocol**

1. Culture cells in a 96 well plate and allow cells to grow for 48 hours.
2. Thaw reagent for about 90 min at room temp
   - a. Ensure the entire vial is thawed before use
3. Pipet 20 µL of reagent into each desired well of a 96 well plate.
   - a. Each well should already have 100 µL of cell/media mixture
4. Incubate the plate for 1 to 4 hours at 37 degrees and 5% CO₂
   - a. 25 µL of 10% SDS can be used to stop the reaction if the plates are then placed in a dark room temp humidified chamber for up to 18 hours
5. Record absorbance at 490nm

**Plate Set Up**

<table>
<thead>
<tr>
<th>Standard Curve</th>
<th>Un-coated nitinol</th>
<th>Chitosan coated nitinol</th>
<th>PLLA</th>
<th>PCL</th>
<th>Control (media)</th>
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</thead>
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**Cell Adhesion Seeding**

- Utilizes fibrin microthreads in procedure, substitute these for wires (coated and uncoated)
- Length used: 2 in
- Diameter: 0.006 in
- Sterilization:
  - Wires washed with 15 minutes PBS
  - 45 minutes 70% ethanol
  - 3 rinses for 15 each in PBS
- V-shaped channel fabrication + cell seeding
  - 2g agar added to 100 ml of DI water (2% solution made) then autoclaved
  - Solution poured into pyramid shaped mold and gels for 45 minutes
  - Template removed, v-shaped agarose chamber transferred to cell culture wells using spatula
  - Culture wells filled with DMEM around v-shaped chamber prior to cell seeding
  - Sterilized wires anchored to bottom of chamber using triangle slabs made of PDMS
  - Cells seeded onto wires and incubated for 4 hours (might vary dependent on cell type)
- Nuclei visualization:
  - Rinse samples w/ PBS to remove unattached cells
  - Stained for 5 minutes with Hoechst nuclear dye, mounted on slide, and viewed under inverted fluorescent microscope
  - Included unseeded wires as negative control
- Cell quantification:
  - CyQuant NF cell proliferation kit
  - Seeded threads trypsinized to isolate cells from thread, followed by centrifugation
  - 50 microLiter solution of 1X CyQuant dye added to each cell pellet and transferred to 96-well plate
  - Plates incubated for 45 minutes
  - Plates read using fluorescence microplate reader to measure fluorescence units in sample wells
  - Unseeded threads used as negative control
References


