Conversion of Human COBE Plateletpheresis Machine for Large Animal Use

Preliminary Report

Biomedical Engineering Design 200/300

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Abstract

The COBE Spectra Apheresis System by Terumo performs plateletpheresis on horses, A one time use tubing kit from Terumo can cost upwards of $2600. The team has been tasked with bringing this cost down to about $100 per use. This can be done in one of three ways: create a simplified tubing design, create a new sterilization protocol for current tubing, or to remake current tubing with new material. The create a simplified tubing design won our design matrix, so this will be the option carried out. Current tubing is made from PVC plasticized with DEHP. However, DEHP has been shown to leach into the blood in this tubing, resulting in accumulation of toxic metabolites. PVC with no DEHP and silicon alternatives are being looked into. Mechanical properties, performance, and sterilization will all be tested to guarantee the new tubing has the same, or better, properties to the original tubing.

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Introduction

Motivation:

Donated blood components are an important commodity in modern medicine. When people donate blood, technicians often process the whole blood to extract components, including cell pieces called platelets. Platelets are involved in many healing processes in the body. Because of this, donated platelets are used in transfusions for patients with defective platelets and platelet injections to speed recovery from injuries [1].

Apheresis is similar to whole blood donation; however, instead of being pumped straight to a collection bag, the blood is continuously pumped through an apheresis device and back into the patient. The device extracts platelets while leaving the rest of the blood intact, so the donor loses less blood than in whole blood donation [1]. Apheresis is known as "plateletpheresis" when used for platelet extraction [1]. Plateletpheresis is used frequently in the United States and is widely considered to be more efficient than whole blood extraction [2]. In human medicine, 75% of 2.5 million platelet doses distributed in the US were derived from plateletpheresis [1,2].

Plateletpheresis has been validated for many animals, including horses and dogs, to obtain platelet-derived materials for animal cell culture or to obtain platelets for animal transfusion [3,4]. However, there are a number of barriers preventing widespread use of plateletpheresis in veterinary medicine. These validations were done using human plateletpheresis machines (COBE Spectra Apheresis System from Terumo BCT) [3]. In equine donors, this produced significantly lower fold increases in platelet concentration than in humans or swine [4]. This is likely due to differences in blood composition, including plasma viscosity [4]. In addition, tubing sets for apheresis devices, including the COBE Spectra Apheresis System are complex, expensive, and single-use [5]. Estimated costs for disposable tubing sets range from $2000-$2600 [6].

This is unfortunate, as a recent study from our client demonstrated the use of equine platelets in a new platelet therapy that could be used cross-species for anti-inflammatory treatments [7]. Equine platelets were used because they have relatively few bloodborne diseases [7]. The model used in this study tested the effectiveness of the platelet-derived therapy for mice with experimentally induced colitis, with favorable results [7]. Inflammatory bowel disease, including ulcerative colitis and Crohn's disease, affects around 3 million adults in the United States alone, impacting a wide variety of populations [8]. The ability to collect high-quality platelet concentrate from equine donors using plateletpheresis will be important for the treatment of these patients as research continues in this field.

Therefore, our team's goal is to develop a method for reducing the cost of tubing in the COBE Spectra Apheresis System, while optimizing the dimensions as much as possible to carry equine blood rather than human blood. We are using the COBE Spectra Apheresis System because it is available to our client and, as previously described, is validated for animal use already.

Existing Devices:

Several apheresis tubing systems have been patented for use in the COBE Spectra Apheresis System. In our research, we focused on patents that describe the tubing structure as opposed to process control methods. There are minor variations on Terumo BCT's tubing sets, one of which will be described here.

- Terumo BCT Extended-Life Plasma Disposable Tubing Set with Leukoreduction Chamber (Figure 3)

This is the tubing set our client would like us to replace or reuse via sterilization. Currently, this tubing set is sterilized once with ethylene oxide and cannot be reused [5]. It includes a leukoreduction chamber, which filters white blood cells out of the platelet concentrate as it exits the centrifuge and locks into sections of the COBE Spectra Apheresis System front panel (Figure 1). One feature that is omitted from later tubing set designs from Terumo BCT is the set of two pump cartridges, which store tubing to lock into pumps [Figure 2, 9]. Newer designs use a single pump manifold for all the tubing; however, this will not fit in the COBE Spectra [10]. We will need to abide by the design of the COBE Spectra Apheresis System front panel and centrifuge so that the tubing will fit without leaks or kinking.

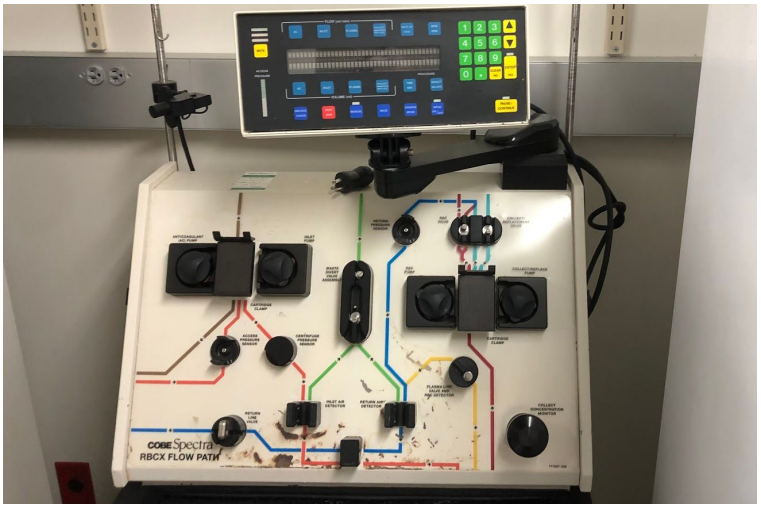

Figure 1: Front panel of COBE Spectra Apheresis System. The colored lines indicate various flow pathways for whole blood, platelet concentrate, and plasma [Photo taken by authors].

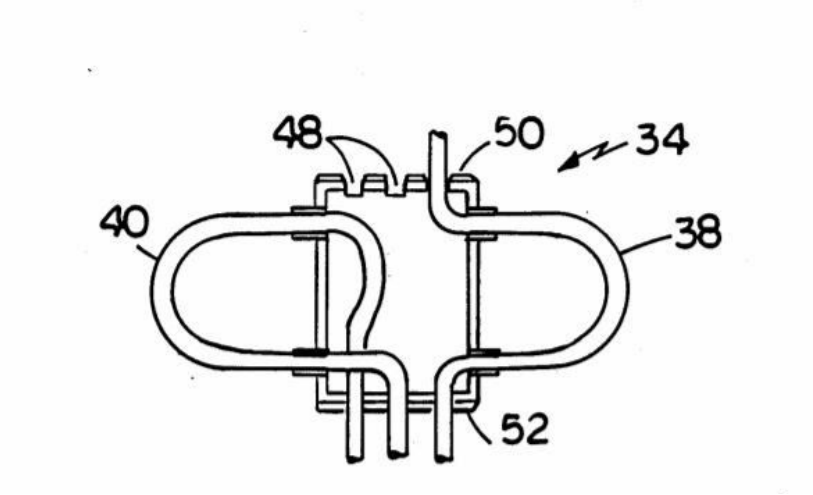

Figure 2: Pump cartridge [11]. This accessory was patented by Terumo BCT (formerly COBE Laboratories). The tubing loops outside the cartridge wrap around peristaltic pumps.

Background

Research:

If an injury occurs that draws blood, platelets are crucial in preventing a bleed out. Platelets release substances, called growth hormones, that promote tissue repair and influence other processes such as inflammation and the immune response. The growth factors that are released from the platelets bind to the healing site and create a gradient that favors the recruitment of stem cells to the site [12]. Knowing that platelets promote healing, it seems like it might be a great idea to create a concentrated platelet concoction and inject it to the source of a wound. That's exactly what our clients and others around the world have been researching.

Plateletpheresis is the collection of platelets from blood. Once these platelets have been collected, blood-derived biological products, such as PRP or Platelet Lysate, can be created. PRP, or platelet rich plasma, is simply concentrating the platelets in the blood. The PRP can then be injected into a patient and the growth factors are slowly released over the period of about a week. Platelet lysate is essentially an immediately active version of PRP. Platelet lysate can be created by taking PRP and freezing it. The ice crystals break open some of the platelets and release the growth factor into the solution [13]. This can then be injected for a more immediate response, as there are growth factors in the solution and the body doesn't have to break down every platelet.

A new type of blood-derived biological product, WEPLEX, has been created by our clients. Our clients are Sabrina Brounts, DVM, MS, PhD, a Large Animal Specialist at the University of Wisconsin, Jacques Galipeau, MD, Director of the Program for Advanced Cell Therapy (PACT), and Andrea Pennati, MS, PhD, is the Associate Director of Research and Development for PACT [14].

WEPLEX an abbreviation for the term washed equine platelet extract. This can be created by taking concentrated equine platelets, washing them, and finally the solution is lysed by a detergent called Triton X-114. This process completely removes platelet materials, such as albumin, fibrinogen and

immunoglobulins, and is 266 times more enriched in platelet derived growth factors than PRP. When WEPLEX was injected into mice with acute tissue injuries, WEPLEX was found to have protective effects against the tissue injuries [15].

The current device used for plateletpheresis is the COBE Spectra Apheresis System. The machine currently uses tubing made from PVC plasticized with DEHP. The machine takes blood from the patient, adds some anticoagulant and saline, and then is run through a centrifuge. The components are then separated into different bags. The platelets are removed and the rest of the blood is returned to the patient.

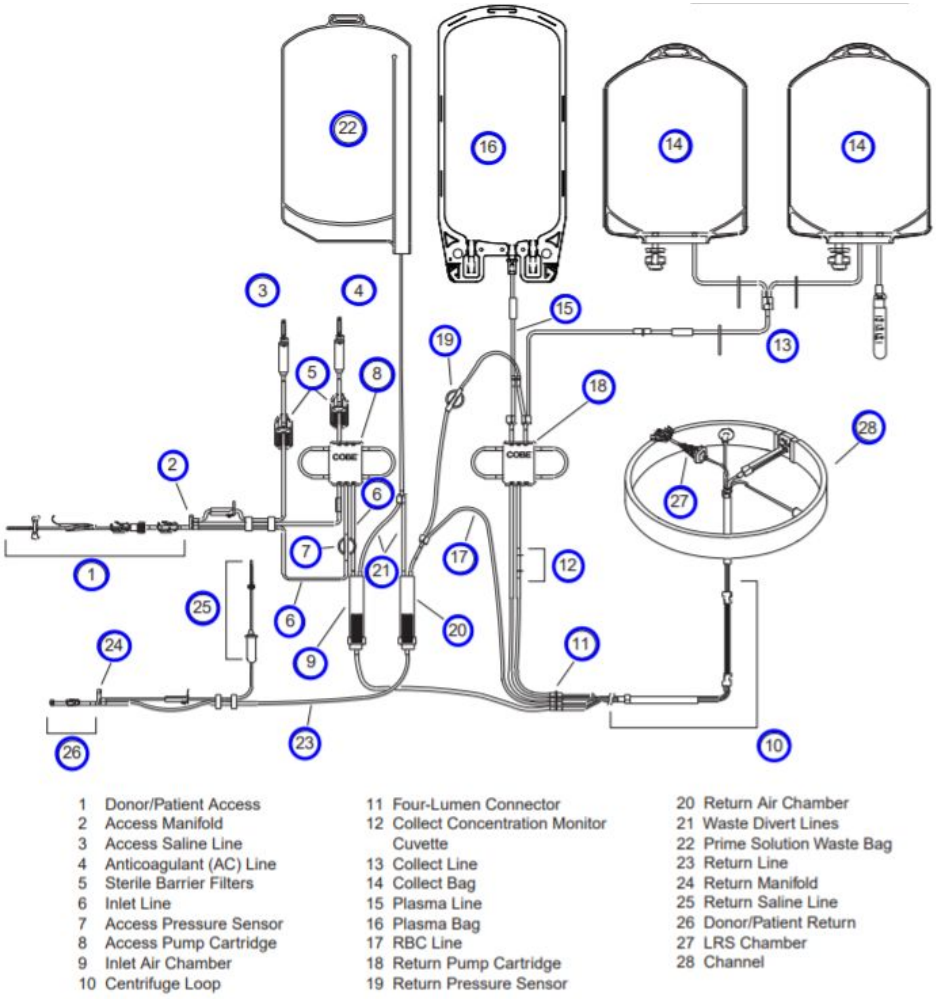

Figure 3: Tubing layout for the COBE Spectra Apheresis System [16]. Some important features: blood comes in at label 1, centrifuging occurs in the circle past label 10, return at label 26, and platelet collection in bag labeled 14.

Design Specifications:

A new tubing must be either bought or fabricated out of plasticized PVC. There is also a possibility of creating a new sterilization technique. Current tubing has a volume of 131 mL [18], outer diameter of 0.16 in +/- .003 in, and a thickness: .022 in +/- .002 in [19]. These measurements result in a total tube length of 756.44 in. The machine should be able to run for at most an hour at a time and have a

max flow rate of 150 mL/min. The main goal is to bring the operating cost down from $2,600 to $100 per use. A more detailed list of design specifications can be found in the appendix.

Preliminary Designs

Simplified Tubing Design:

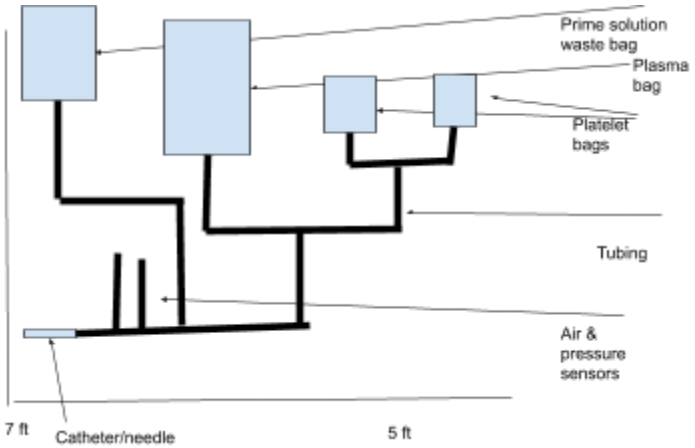

Figure 4: Simplified Tubing Design. This image shows an idea of what simplified tubing would look like.

Our first design idea is to create a simplified version of the tubing that is cheap to produce and would be disposable. We are planning on using the material silicone for tubing because it is chemical and temperature resistant. Our client has needles and catheters. We would buy components such as filters, collection bags, plasma bags, and different sensors separately and then assemble them together. We have a meeting scheduled with our client to talk about the necessary components of tubing and the components that can be simplified.

Sterilization Protocol for Current Tubing:

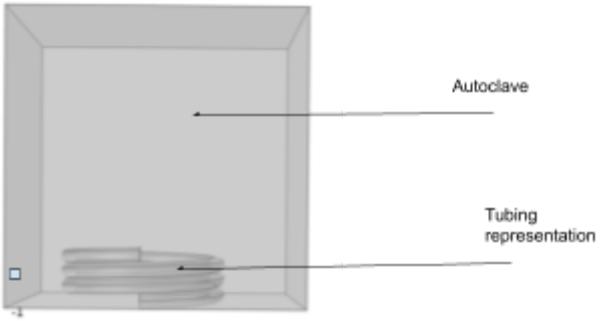

Figure 5: Sterilization Protocol for Current Tubing. This image shows a representation of what the tubing would look like in an autoclave. It is not drawn to scale.

The second design idea is to sterilize the current tubing to make it reusable. There are two types of sterilization we considered: steam sterilization [19] and chemical sterilization [20]. Steam sterilization exposes objects to direct steam at a high temperature and pressure for a certain amount of time. Gas sterilization uses ethylene oxide to disinfect the material. We would use steam sterilization because it is faster, cheaper, and more accessible. In addition, ETO has been shown to be toxic to animals. Although during gas sterilization the material undergoes aeration to remove ETO, there is still concern that residual ETO will remain in the long tubing.

Remake Current Tubing With New Material:

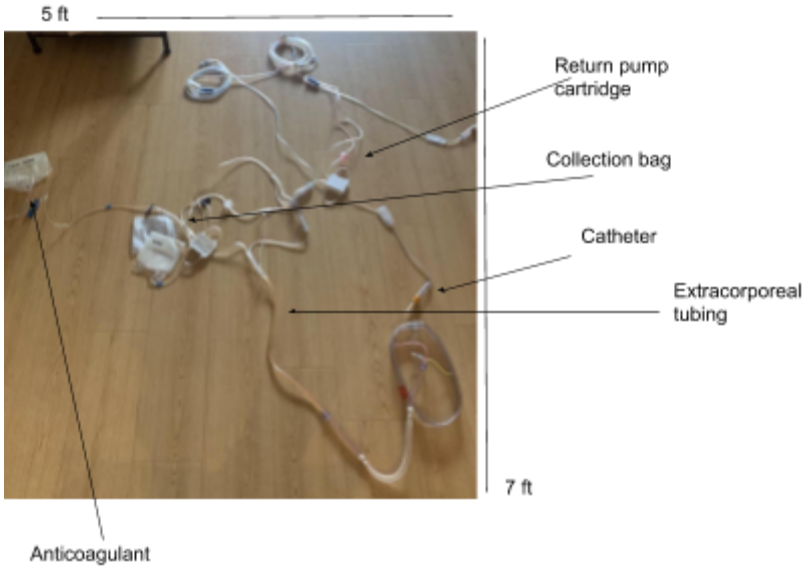

Figure 6: Current tubing. This image shows the tubing from Terumo given to us by our client that we would recreate exactly.

Our final design idea is to buy components of the tubing separately and then assemble them together to avoid paying an inflated price. We would not make any simplifications but would instead follow the specifications of the COBE Spectra Apheresis System to recreate the tubing exactly based on the tubing our client gave us and information available on the Terumo website. Following this method, we would know exactly how the tubing interfaces with the machine and we would have a guide to follow.

Preliminary Design Evaluation

Preliminary Design Route:

We discussed with our client about the final deliverable product that was required for the completion of this project. The client informed us of multiple possible avenues that we could take. We were informed that alongside tubing the machine needed to be reparameterized to support a large animal. Currently the machine is set for a large human. The apheresis machine needs rate in and out changes that need to be

done via the software. They also wanted calculations for the maximum amount of blood that could be drawn out and processed within an hour of time. After discussing our clients and getting an understanding of the various aspects of the whole project, the team has decided that it would only be feasible to complete one aspect within the semester. The team has concluded we will move forward with creating a cheaper to use set of tubing and allow for future students to complete the rate in and out programming.

Design Matrix:

After deciding on the tubing aspect of the project and coming up with preliminary design ideas, we created a design matrix (Table 1) to aid in the evaluation of our designs and allow us to choose design to pursue.

Table 1: Design Matrix Evaluating Three Design Options to Create a More Effective Tubing

		Simplify cheap tubing		Sterilization protocol for current tubing		Remake cheap tubing	
Criteria	Weight	Score (max 10)	Weighted Score	Score (max 10)	Weighted Score	Score (max 10)	Weighted Score
Cost per Use	26	9	23.4	10	26	6	15.6
Sterility	21	10	21	7	14.7	0	0
Safety of patient	19	8	15.2	10	19	7	13.3
Durability	18	7	12.6	6	10.8	6	10.8
Ease of Fabrication	10	7	7	8	8	10	10
Ease of Use	6	10	6	6	3.6	1	0.6
Sum	100	Sum	85.2	Sum	82.1	Sum	50.3

Six criteria were utilized to determine the best design option each with a different weight that corresponds to the importance of said criteria. The first and most important criteria is cost per use. The client expressed to us that the main reason she came to us is because the tubing was too expensive for her to justify in her research. The design aspects of the tubing should remain fairly similar, but the cost of fabrication and use needs to become more efficient. The next two criteria fall under patient care. Sterility and safety of patients are always important when considering any device involving bodily fluids. The tubing must be safe for the patient and cannot risk contamination or leaks. It also must be sterile so the red blood cells can return into the body without risk of spreading disease. Ease of fabrication is an aspect to be considered especially given the current situation. The pandemic restricts our access to each other and fabrication areas so having a design that can be produced on campus by only a few people is key. Ease of use was considered since the tubing will be removed and used multiple times. We want the tubing to not have advanced connectings, but this is ranked low considering trained professionals will be operating the machine and they will have more knowledge behind the attachment of tubing.

The simplified tubing and sterilization of the current tubing ranked highest in cost per use. This is because the current tubing is quite expensive so an overall new tubing would prove to be cheaper. However, the client already owns several sets of the current tubing that they acquired for free. If there was a way to gain several uses with the current tubing then the cost per use would only be the sterilization

cost. The client has yet to specify how many uses they need but ten uses per tube would give the client plenty of platelet-rich plasma for their research.

Sterility is best in the simplified tubing model because we could choose a material that can be easily sterilized. This is the reason it beat a sterilization protocol for the current tubing. The current tubing's material cannot handle autoclaving temperatures and we'd have to settle for a less effective sterilization method. A simplified tubing would allow us to choose materials that are intended for multiple use after sterilization. This aspect will ensure a 100 percent sterile tubing after each use.

All of the designs rank within points of each other when it comes to the safety of the patient. This is because the tubing is away from the patient and the only interaction that the patient will have is when the blood re enters. That is covered in sterility. The leaking of blood may be hazardous to the patient, but a selection of sealed tubing material will negate that issue. The reason the sterilization protocol edged over the others, is because the tubing was built to be used by patients and has FDA approval giving it an added boost in safety.

When it comes to durability, simplifying the tubing with new materials ranks the highest since we have the freedom to choose the material strength. The current tubing has a fixed durability and is pretty good, however, it's only made to last one time so the manufacturer can make money. The cheaper tubing will not provide great durability as we will sacrifice a lot of structural integrity trying to find cheap materials and also optimizing too much will leave holes in the tubing.

Fabrication may prove to be difficult across the board, considering the tubing is so complex. A sterilization protocol may seem like the easiest to fabricate, but we'd have to include a separation within the tubing that would need to occur and then reattaching the tubing into a singular set again. This led us choosing the remaking a cheap tubing design as the easiest to fabricate. We could essentially cut pieces and use adhesive to put them together. After each use they would be thrown away so no assembly required.

A simplified tubing design would allow for the easiest of use. The current tubing requires professional training, but a simpler design would allow for less experienced operators. The cheap tubing may prove to be difficult to use considering the fabrication of the tubing will be dependent on use. After each use we would have to make a new one or give instructions to the client. This would then require them to learn yet another protocol adding unnecessary work for them.

Purposed Final Design:

The team weighted and scored each criteria and the highest scoring design idea was the simplified cheap tubing. We have decided to pursue a new material that would better suit our needs and simplify our current tubing. The team also decided to keep sterilization in mind. We have proposed to create a simpler version of the tubing, but make it slightly more expensive than originally planned. It would still be under the cost of the current tubing, but the price could be justified on its ability to be sterilized and reused. The cost per use would still be kept under the clients requirements.

Fabrication/Development Process

Materials:

- According to a Terumo representative, their apheresis tubing is made of PVC plasticized with DEHP (di(2-ethylhexyl) phthalate). However, DEHP has been shown to leach into the blood in this tubing, resulting in accumulation of toxic metabolites [6]. For a new tubing system, we will consider DEHP-free PVC tubing.
- In addition to plasticizer toxicity, the material must be shown to retain ethylene oxide residuals below acceptable limits after sterilization, resist degradation by high-pressure steam, or be significantly cheaper than currently available apheresis tubing. Plasticized PVC would be ideal due to its flexibility and resistance to kinking, but other clear, flexible plastic alternatives include liquid crystalline polyesters, thermoplastic elastomers, or PTFE (mainly for collection bags) [7, 8].
- Silicone is another consideration for fabrication, it has been shown to withstand autoclave sterilization and maintain its existing structural integrity. [21]
- At this time, it seems that silicone is a better fabrication material for autoclaving sterilization and while PVC is a bit better material for ethylene oxide sterilization, silicone is a better choice as it is most tolerable of both possible sterilization techniques.

Methods:

- The current tubing set consists of 28 parts (refer to Figure 3), once the client has specified which parts can be removed for safe use on a horse, the existing set can be simplified and a detailed graphic can be made to be sent to the third party contractor that is selected for fabrication.
- Current conditions with third party contractors are as follows: Trevor is in contact with the company Cole Parmer through personal connection and they will only fabricate tubing in 10,000 foot increments. The mould would cost $250 to $300 up front. The tubing can then be sold in either sterilized bagged increments, one large spool or multiple smaller spools. This may not be ideal depending on how many of the 28 previously mentioned tubing components need to remain in the final design.

Final Prototype:

To be updated at a later date.

Testing

The tubing must be flexible enough to withstand compression by the peristaltic pumps in the device and keep from kinking in the centrifuge. The seals must be able to contain fluids, including saline solution, anticoagulant solution, and horse blood. Finally, our sterilization protocol should be validated to show that it can safely sterilize the tubing set.

Mechanical Properties: Because we plan to buy medical-grade tubing, testing material properties such as necking, elastic modulus, and durometer will not be necessary. However, it is important to test the tubing for kinking because tubing in the COBE Spectra Apheresis System is subject to repeated clamping, bending within the centrifuge chamber, and compression by peristaltic pumps.

A basic test for resistance to kinking measures the minimum bend diameter [22]. This is found by making a loop from the tubing and tightening the loop until the tubing wall collapses [Figure 7]. The diameter of the loop before kinking is the minimum bend diameter. Currently available flexible PVC tubing sets have a minimum bend diameter of 1.5 cm [22].

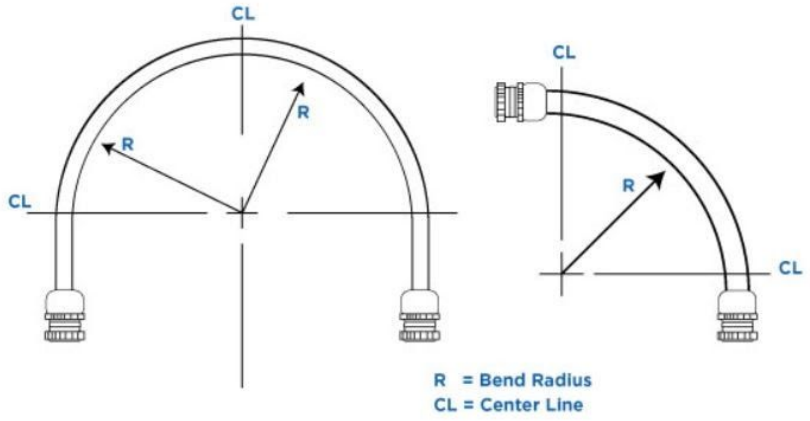

Figure 7: Example of a minimum bend diameter/radius test [23]. This test is used to examine how resistant a tube is to bending.

Performance: FDA guidelines for hemodialysis tubing require a set of performance tests to ensure reliability. Because apheresis tubing also carries blood pumped through an extracorporeal circuit, these guidelines can be used for our testing.

Our proposed performance testing strategy will use the COBE Spectra's Priming function, which flushes the tubing set with saline and begins turning the pumps [24]. Once the tubing is inserted into the device, the initial test will make sure that saline does not leak out of any segments. If this test is completed successfully, we will move on to testing the flow of an equine blood-mimicking substance. A study from the London Metropolitan University demonstrated that a substance with similar dynamic properties to equine blood can be made with flour, glucose, glycerol, bovine serum albumin, red food coloring, and water [25]. The COBE Spectra Apheresis System can be primed with this substance to mimic horse blood.

During this testing, the tubing should be clamped repeatedly to ensure that it can close off completely without kinking. According to FDA guidelines, each fluid test should be done over 4 hours at 37°C, inspecting tubing periodically for kinking [26].

Sterilization: Vacuum autoclave cycles, which use vacuum pulses to pull air out of the chamber and the contents, are useful for medical tubing [27]. This can be tested using commercially available strips or discs of bacterial spores (*Bacillus atrophaeus* and *Geobacillus stearothermophilus*) [28]. The discs are small (0.25 in) and can be placed inside one end of the tubing (0.113 in diameter) during sterilization, or suspensions of spores can be used to inoculate the tubing interior [28]. Incubating the discs and exposing them to commercially available reagents give a colorimetric pass/fail test in accordance with current ISO sterilization standards [28].

FDA guidelines recommend tests for pyrogens, or fever-inducing endotoxins, as well, citing the limulus amebocyte lysate (LAL) assay as an example [26]. Although these are usually done in dry heat sterilization, it is possible to test pyrogenicity from bacterial endotoxins in moist heat sterilization [29]. It is not clear if we will have the budget for this assay in addition to spore tubes and may pick one depending on our clients' preferences.

Discussion

This project will have global implications because it will improve the use of platelet lysate in anti-inflammatory and regenerative therapies. Our project will allow our clients to extract platelets from horses, using their novel technique of concentrating, washing, and then lysing platelets with the detergent Triton X-114. Platelet lysate contracted by this method has 266x the growth factors as platelet lysate derived from the traditional freezing and thawing of platelet rich plasma [7]. In addition, it is devoid of plasma proteins which can cause serum sickness and allergic reactions. This has the potential to make huge gains in the field of regenerative method.

The main ethical consideration with this project is its use on horses who cannot consent to experimentation. However, we are confident that Dr. Brounts is an expert in equine care and that the horses will be safe and well treated. In addition, since horses are large and have 50-60L of blood they will be able to withstand the procedure.

As a result of further evaluation we may decide to change the diameter of the tube to increase or decrease the flow rate. We will determine this after further testing. We also foresee possible difficulty and error in interfacing our designed tubing with the COBE Spectra Apheresis System by Terumo. Since our tubing is not the standard tubing that matches with the COBE Spectra Apheresis System this is something that is unavoidable but that we will work out.

Conclusions

The client Dr. Sabrina Brounts requested we make the COBE SPECTRA Apheresis Device more cost effective for her research. She would be using the device on horses, except she cannot justify the cost per use of 2000-2600 dollars. This cost mainly comes from the single use sterile tubing set. Each set costs roughly that amount, except a new problem occurred when the manufacturer of said tubing set announced they will no longer make the tubing. Dr. Brounts now needs a new cheaper set of tubing that will perform the same tasks so she can have access to the platelet-rich horse plasma she uses for her research.

After research, meetings, and discussions the team has decided to pursue the option of designing a simplified cheaper version of the existing tubing that can support sterilizations via chemicals or autoclave. The simplified cheaper tubing, will consist of a non-medical grade tubing, that can handle high temperatures and exposure to biomaterials. The tubing itself will not include some of the human safety measures that are not required when dealing with a horse. The whole new set will need to be assembled from different tubing diameters and lengths to resemble the current model.

The project as a whole will have societal benefits in the medical industry. Dr. Brounts research with platelet lysates has the potential to lead to ground breaking discoveries in regenerative medicine. The conversion of the COBE apheresis device will allow Dr. Brount and her fellow researchers to have easier access to the platelets they study.

Our team was able to successfully address the issues brought to our attention by the client. However, there were aspects of the machine that needed improvement that we could not accomplish. Nonetheless, we found that we could comfortably solve the problem of tubing, which will allow Dr. Brounts to extract blood. Future students can research and fix the problem of blood extracting rates and time efficiency. In terms of difficulty, we ran into obstacles regarding the tubing and how complex it was. We also struggled to understand how the tubing fit into the machine and there were some communication issues with our clients needs and what we are capable of doing within a semester. Given the opportunity to do it again, the team agrees that a meeting with all of the clients needs to happen sooner. We also need to get a hold of the current tubing right away while also getting reliable access to the COBE machine.

In the future we hope to move forward with a specific material that will satisfy our product design specifications. In addition, we need to meet with the clients to discuss aspects of the tubing set that can be removed completely from the design. The COBE machine itself must be moved to a more accessible location where we can study how the current tubing runs into the machine, and make notes as to what specifics we need to keep in mind when it comes to our tubing. An effective method of separating the current tubing into its individual parts must also be determined.

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Appendix

A. PDS:

Product Design Specifications

BME 200/300 - VETMED: Conversion of Human COBE

Plateletpheresis Machine for Large Animal Use

09/17/2020

Clients: Professor Sabrina Brounts, Dr. Jacques Galipeau, and Dr. Andrea Pennati

Advisor: Dr. Melissa Kinney

Team Members: Aditya Ailiani - Team Leader (300), Cate Flynn - Communicator (200), Nesya Graupe - BSAC (200), Lokesh Kumaravel - BPAG (200), and Trevor Silber - BWIG (300)

Function: Plateletpheresis uses centrifugal technology to separate platelets from other donor blood components, returning other components to the donor. Our clients, Professor Sabrina Brounts and Dr. Andrea Pennati, have developed a new equine platelet therapy and would like to use plateletpheresis rather than whole blood extraction to extract platelets for further research [1]. The COBE© Spectra Apheresis System they are using costs $2000 - $2600 per use, mainly due to the expensive tubing sets that can't be reused. Our goal is to develop a system to decrease cost per use, allowing the client to reuse tubing sets or use cheaper tubing.

Client requirements:

- Replace all of the tubing in the COBE© Spectra Apheresis System by Terumo
- The operating cost should go down from $2300 a use to about $100 for a reusable tubing system and around $20 for a disposable single-use tubing system
- As a final deliverable, the client would like either a cheap set of tubing, a new tubing fabrication technique, or a new sterilization process and calculations for optimal machine settings and flow rate calculations for extraction from a horse over an hour.

Design requirements:

1. Physical and Operational Characteristics

a. *Performance requirements:*

- Single Use Condition: If the tubing can be produced at a low cost, the client has specified that a single use tubing system could be adequate. In this situation, the tubing would be disposed of after every use. This approach would remove durability concerns from use to use, but there are no cost estimates at this time to decide if this is a viable approach. ($20 cost estimate)

- Multi Use Condition: If the tubing can be produced from a more durable material, the client has outlined that they would be satisfied with 20 to 25 uses with sterilization between uses. ($100 cost estimate)
- Sterilization Condition: If the original tubing is kept, the client has stated that they would be satisfied with the development and documentation of a comprehensive sterilization of the existing tubing (most likely by gas or steam, autoclave or ethylene oxide). If this approach is pursued, there will need to be testing to determine the durability of the existing tubing, as it has only been used once in the past.

b. *Safety:* The product involves the transportation of animal blood, therefore standard biochemical safety regulations apply as outlined in the Laboratory Safety Guide by OSHA [2]. The original tubing is medical grade and follows regulations in place for human consumers, but animals tend to have fewer regulations, since bloodborne pathogens are less common. In order to protect the handlers of the product, the tubing must be non-permeable to restrict leakage of the blood. Biohazardous contamination is the main safety concern when working with the tubing. We may want to consider adding a biohazard indication after every use of the product until sterilization is complete.

c. *Accuracy and Reliability:* The tubing should withstand being washed and reused 40-50 times. Any malfunctions will likely put the horse in danger and therefore the tubing must successfully collect blood from horse, transfer to apheresis machine, and return to horse every time used with no clotting, breakage, stoppage, or other difficulty.

d. *Life in Service:* The client requested tubing that can be reused at least 20 times (more reusable would be better), so the material would need to withstand either repeated steam or EtO sterilization without degrading or accumulating EtO residuals. Equine plateletpheresis has a run time of approximately 160 min, so the tubing should withstand working pressure at least that long [3].

e. *Shelf Life:* The tubing, bought or fabricated, will be created in a sterile environment and placed into a pathogen free sealed container. Nothing from the outside environment will be able to get in, meaning the tubing will stay sterilized. The tubing should be kept out of direct heat to prevent any possible deformation. Theoretically, the tubing should be able to be stored for an indefinite amount of time.

f. *Operating Environment*:
- The tubing and machine cannot be exposed to temperatures above 81° F.
- The inlet flow rate cannot be 25 mL/min or less.
- The tubing cannot be occluded or bent before use, this will damage the machine.
- Any stretching of the tubing will result in damage to the tubing itself or the machine if it has already been connected.
- Tubing must be stored in a sterile environment: outside contaminants such as hair or dirt will damage the machine or contaminate the extracted platelets.

- The tubing must be dry upon use: any excess fluid can cause an unwanted electrical response.
- The tubing should only be handled by this team in testing or the client and their approved partners when finished. [4]

g. *Ergonomics:* The plateletpheresis device will have set limits on the rate of blood draw and transfusion. Although larger animals can handle a faster draw rate, the client has asked us to keep the limits preset on the machine. The inlet pump rate will have a maximum restriction of 65 mL/min for the Dual-Needle procedure [4]. Tubing will interact with the larger animal's blood, specifically a horse. Equine blood is on average 100° F, therefore the tubing must be able to handle such temperatures [5].

h. *Size:* Tubing should be portable and, when gathered together, not larger than 2ft x 2ft. Tubing should contain a 1L bag to hold collected platelets [3]. The length of tubing required will be determined once we see the setup of the apheresis machine and proposed area where the horse will stand. The tubing should be very accessible for cleaning. The tubing itself should have a diameter of approximately 0.115 inches and hold 150 mL of blood [4].

i. *Weight:* Tubing weight is not a major concern, as the diameter and length of the tubing are fixed. Once we see the tubing, we will be able to record specifications for weight of the plastic they use; any tubing we buy or make should be similarly lightweight for the user's convenience.

j. *Materials:*

- According to a Terumo representative, their apheresis tubing is made of PVC plasticized with DEHP (di(2-ethylhexyl) phthalate). However, DEHP has been shown to leach into the blood in this tubing, resulting in accumulation of toxic metabolites [6]. For a new tubing system, we will consider DEHP-free PVC tubing.
- In addition to plasticizer toxicity, the material must be shown to retain ethylene oxide residuals below acceptable limits after sterilization, resist degradation by high-pressure steam, or be significantly cheaper than currently available apheresis tubing. Plasticized PVC would be ideal due to its flexibility and resistance to kinking, but other clear, flexible plastic alternatives include liquid crystalline polyesters, thermoplastic elastomers, or PTFE (mainly for collection bags) [7, 8].
- Silicone is another consideration for fabrication, it has been shown to withstand autoclave sterilization and maintain its existing structural integrity [13]

k. *Aesthetics, Appearance, and Finish:*

- Since the final product is a component of an existing machine, it will need to conform to the existing shape of the current tubing system. The inner diameter is 0.113 in and the total volume is 131 mL (more specifications can be added when current tubing is acquired from the client) [4].The tubing will be clear as the appearance of the inside of

the tube will be one parameter in judging the cleanliness of the tubing after sterilization if a multi use tubing system fabrication is pursued.

2. Production Characteristics

a. *Quantity:* The quantity of the product depends on the frequency of use. The product cannot be used indefinitely and the tubing will need to be replaced after x amount of uses. The goal is to improve the current tubing so that it is cheaper and is more durable for more uses after sterilization. Ideally, the quantity of tubes being used will be kept to a relative minimum throughout the life of the COBE Spectra Apheresis System.

b. *Target Product Cost:* The target cost is less than $100 for reusable tubing and around $20 for single use tubing. Terumo Blood and Cell Technologies currently sells disposable tubing for the COBE Spectra Apheresis system for $2,600 a use.

3. Miscellaneous

a. *Standards and Specifications:*

- *Specific to device:* The COBE Spectra Apheresis System that the client is using is Type B medical electrical equipment according to IEC Standard 60601-1 [4]. This means that the parts that come in contact with the patient are non-conductive [9]. Because we are not making changes to the electrical components of the Apheresis System, the precautions taken by the manufacturers for insulation and protective earthing will be sufficient [9]. The device itself was unclassified by the FDA but was subject to 510(k) premarket notification [10].

- *Sterilization:* Under IEC 60601-1, multi-use equipment that contacts patient bodily fluids must include detailed instructions for sterilization including parameters such as allowable temperature, pressure, humidity, and exposure time [9]. These parameters will depend on the tubing material and must follow validation requirements in ISO 11134 (autoclaving) or ISO 11135 (ethylene oxide) [9]. Current COBE disposable tubing sets are sterilized with ethylene oxide before use once and cannot be used again, likely due to retention of biohazardous EtO residuals [4, 11]. A modified sterilization technique or alternative tubing system would have to effectively kill resistant microbes (as described in ISO 11134,11135) and retain EtO residuals below the maximum safe levels outlined in ISO 10993-7 (Biological evaluation of medical devices — Part 7: Ethylene oxide sterilization residuals) [11].

- *Tubing:* The FDA classifies blood collection devices, including tubing, as Class II medical devices, making them subject to premarket notification [12]. The tubing should be flexible enough to avoid kinking, which can result in clotting in the line [4]. The

tubing should withstand pressures at least from -250 to +400 mmHg, and have about 0.113 in. inner diameter, and be made of a clear plastic similar to PVC based on the COBE Spectra device's standards [8, 12].

b. *Customer:* Currently, the product will not be sold on the market, just used in our clients laboratory space. However, Terumo has been phasing out many of the products for the COBE© Spectra Apheresis System, as there are newer machines on the market. A new tubing could be sold to others who also have this machine. If a new sterilization technique is created, this can be applied to any range of products that need to be sterilized after coming into contact with blood.

c. *Patient-related concerns:*

- The intended use of this product is on horses. This indicates a lower concern for protection of patient data, it is likely that any horses used in testing or final intended use will be assigned subject numbers.
- There is a lower concern for bloodborne pathogens in horses, but sterilization between uses will still be required. Ethylene oxide is currently used as a sterilization agent. [4]

d. *Competition:* We are tasked with finding a more affordable and easy to clean tube that is already on the market. The tube must fit our clients needs. The likelihood that we create a new type of tubing is slim, therefore, we do not have to be wary of patents. As far as competition, there are hundreds of medical and non-medical grade tubing options and we are not necessarily competing with any of them.

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B. Materials List:

Material	Part Numbers	Place Purchased	Cost	Quantity	Budget
COBE Single Use Tubing Set (Current model)	Set is pictured in Figure 3	Donated by clients and picked up from Dr. Pennati	Free	1	$500
10, 12 & 14 Gauge Leads and Catheters (To be used to collect blood from horse)	2 x 10 Ga Catheter 2 x 12 Ga Catheter 5 x 14 Ga Leads	Donated by and picked up from Dr. Brounts	Free	4 Catheters 5 Leads	$500