Saving Limbs: A Way to Salvage Severed and Partially Severed Appendages

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Abstract:

This project's objective was to create a device that enables efficient cooling of limb tissue in situations with traumatic amputations for successful reattachment. The device was required to cool and maintain the limb at temperatures between 16-18 degrees Celsius for at least six hours while also being lightweight and portable. By ensuring the device met these requirements, the overall viability of the limb was improved allowing for a higher chance of successful reattachment. To accomplish effective cooling, liquid carbon dioxide was used. Designing a prototype consisted of iterating through ideas that would allow the CO2 to be pumped from a siphoned liquid CO2 tank through a tubing system into the device. The final prototype was built using a vinyl mesh dry bag, an emergency blanket, neoprene foam, and two one-gallon plastic vacuum-sealable bags attached to a tubing system. To test the viability of the device, a 2-liter bottle of Jello and a pig leg were used as surrogate limb. Temperature probes were placed in the center and on the surface of the surrogate limbs. The limbs were heated to body temperature and placed inside the device. The limbs were heated to body temperature and placed inside of the device with the goal to achieve an internal temperature of 16 and 18 C. Longevity testing was also completed at desert like temperature to test the device's ability to cool and maintain our target temperature under a six-hour period. Ultimately the device was able to cool the pig leg in 220 minutes while only using ~5.3 lbs. of liquid CO2.

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Chapter 1: Introduction

Cooling is used in several medical applications to preserve the physical and mechanical properties of tissues. One primary application is during limb transportation after a traumatic amputation occurs. Currently, the process includes placing the severed limb in a cold environment, typically a cooler or a bag, that is surrounded by ice. This method is not effective enough given the severity of the situations in which it is used. More specifically, when it comes to larger limbs, having these types of resources such as ice and a large enough carrying container is not always feasible. This project aims to create a new, sterile, portable, lightweight, and effective method for transporting traumatically amputated limbs. The aim is to preserve larger limbs such as arms and legs. Traumatic limb amputations have accounted for about 45% of all limb losses in the United States [1]. Common causes for this include military explosions, traffic accidents, and industrial machinery accidents.

According to Pro Medical East, over 1 million amputations occur globally. An average of 45% of civilian amputations occur due to car accidents or other traumatic accidents. In war zones, an average of six out of 100,000 soldiers loses their limbs in combat. Other incidents such as work trauma and safety result in over 20,000 amputations a year. On average, 83.5% of limbs can be salvaged if transported and treated properly. This is why it is important to have a proper means of limb transportation to the nearest medical facility to salvage the limb. Currently, the amputated limb is wrapped in saline solution and put into an ice bag. It is still not known why this is the state-of-the-art solution. However, it can be because cold temperatures decrease inflammation and maintain homeostasis by constricting blood vessels which constrict blood flow. Even though medical professionals have addressed that this is not the best method, it is still the preferred method of preservation. With that being said, 65.9% of limbs are not preserved correctly which decreases the chances of reattachment. In cases of war, the nearest hospital might be at least three hours away. A limb in an ice bag would lower in metabolic rate even though there is a sever loss in nutrients and blood flow. Therefore, it is vital to find a different solution that can preserve the limb longer without losing its metabolic functions.

Current methods of limb preservation following severe injury do not provide sufficient successful rates of reattachment. The team aims to create a solution that will compete with and exceed today's standards. Severe limb injuries resulting in partial or complete severance require immediate attention to maintain viability for reattachment. To account for most limb injuries, the team will create a design that is able to be used in any location, situation, and body. The design will be portable, temperature resistant, used by medical professionals, and biocompatible. The main target of the device is to decrease metabolic rates while minimizing ischemic times to overall reduce the amount of tissue necrosis, specifically in the muscle. To accomplish this, the team will analyze the current methods of preservation and determine what works and needs to be improved, as well as if it is targeting the underlying cause, or just putting the limb in suspension. A design was then developed, considering current solutions as well as other biomedical applications in other parts of the body that may apply to this scenario.

The final design addresses minimizing tissue necrosis and promoting limb preservation through cooling technology using liquid carbon dioxide. The device mimics a flexible yet structured cylindrical casing that slides over the site of the injury with inner components. Within the casing, there will be a 360-degree space for the liquid carbon dioxide to be pumped into, causing it to turn gaseous and even into dry ice snow. This will then settle on an inner sleeve, composed of a non-woven fabric to protect the limb from direct contact with the ice, while still allowing peripheral cooling from the gaseous carbon dioxide. Within the inner sleeve, resides where the limb with be housed. Before placing the limb into this area, it will be wrapped with sterile plastic to contain biohazardous fluids as well as a gel layer that will allow for further cooling of the limb. By having this gel layer, the limb will be able to hold onto the cooling provided by the carbon dioxide a bit longer. The entire device will be secured with drawstring technology

to ensure a snug fit around the limb in cases of partially severed limbs. This design will advance current state of the art technology by providing a consistent cooling source to the limb during long transportation times and can allow for temperature regulation to ensure the limb remains at the target temperature of about 16 degrees Celsius.

This paper will begin by addressing the literary background and information necessary to understand the motivations of the project. This includes considering the blood flow specifically in veins and arteries, the types of tissues affected, and the types of amputations that typically occur. The situations of these traumatic amputations will also be discussed, more specifically regarding the population affected, the area and limb amputated, and the causes of these amputations. Lastly, the background will conclude with a review of current methods and possible new methods for preserving these fully or partially severed limbs. The paper will progress onto the approach being taken on this project. It will outline the objectives and constraints, the needs, and the assumptions needed in creating a new solution. The design and specifications of the chosen design will follow, including all processes for creating, modeling, testing, and finalizing the design.

Chapter 2: Literature Review

When researching ideas for a novel medical device it is important to understand the intricacies of the problem. The literature review chapter of this report provides a comprehensive exploration of traumatic amputation and its aftermath. Starting with the blood vessels, their role in ischemia was investigated to understand the impact a traumatic amputation has on various body systems. The review continues into exploring the types of amputations—crushing, avulsion, and laceration— and in what situations they occur. When, why, and who these types of amputations occur most prominently were also analyzed to best design the device for the population, environment, and situations where the device would be used in real-world scenarios. Existing solutions and upcoming solutions were reviewed including the state-of-the-art treatment of using ice-water. Failures of current solutions ultimately result in lower success of replantation surgery and thus, the novel device design explored in this paper aims to be more successful than its predecessors.

2.1: Pathology

At a basic level, arteries move oxygenated blood away from the heart and veins bring deoxygenated blood back towards the heart. Nerves are essential in controlling the minute changes in blood pressure throughout the circulatory systems. When a limb is amputated, the nerves are severed. This results in the blood vessels starting to spasm, retract back into the amputated limb, and ultimately shrink [7].

Ischemia is defined as a lack of blood flow or very poor blood flow to an area of the body. There are several situations where ischemia could occur, and it is not limited to severed or partially severed limbs. Myocardial ischemia is an example of when ischemia occurs as a response to changes in coronary perfusion (flow) [8]. An example specifically related to limbs is acute limb ischemia (ALI). When ALI occurs in the arms or legs it can cause further medical issues such as tissue injury, diseases, or sometimes even death [9]. Depending on the affected limb, the root cause of ALI can be situ thrombosis for lower extremity cases. For upper extremity cases, an embolism can cause this ischemia. There are also various parts of the body that can withstand ischemia and parts that do not respond well. Bone, tendons, fat, and skin tend to survive with prolonged ischemia, while muscular, vascular, and even neural tissue have a low tolerance to ischemia and are affected to the point of loss of function [10].

The effect of ischemia on tissue depends on the area of the body and the type of tissue being affected as well as temperature and ischemic time. In skeletal muscle, for example, the critical ischemic time at a normal body temperature was 4 hours. For nerve tissue, 8 hours was reported, 13 hours for fat tissue, 24 hours for skin tissue, and lastly 4 days for bone tissue [11]. Another factor that affects the ischemic time is the type of muscle fibers, where type II muscle fibers are more resistant to ischemia. Ischemia time plays a large role in the viability of the limb for reattachment. Once the limb is severed, completely or partially, the care of the limb is critical to determining the outcome and preventing cell decomposition. Ischemia occurs in either warm (normal temperature) or cold conditions. Warm ischemia is typically when the limb is left at ambient temperature. Cold ischemia occurs when the limb lacking blood supply is cooled/chilled. Each has different benefits and drawbacks and may depend on the environmental situation. The recommended time for each may depend on the severity and location of amputation. For example, recommended warm ischemic times for digits is up to 12 hours, whereas this time gap shortens significantly with major limbs down to 6 hours. For cold ischemia, we similarly see the drop-in time as recommended cold ischemic times for digits are 24 hours and drop to only 12 hours for major limbs [12]. Within each type of injury, different tissues can withstand varying times. Regardless of the maximum time recommended, it is important that the limb is properly handled to prevent further necrosis and cell degeneration.

Muscle is important for performing basic functions in everyday life. Following severe injury

resulting in severance or partial severance, the muscle in the limb ceases to obtain proper blood supply. Lack of blood can cause damage to different muscle fibers that are responsible for movement. Type II muscle fibers, known as “fast-twitch”, suffer from larger amounts of necrosis and decrease in overall fiber amount in comparison to type I (slow-twitch), responsible for contraction and posture [11]. Type II fibers are predominately found in the upper arm muscles, such as the triceps, whereas type I fibers are found more in the leg muscles [13]. This makes sense as legs contribute greatly to support of the body, where arms are more locomotive and responsible for a greater range of motion. Muscle tissue death may also be greatly impacted using a tourniquet [14]. Prolonged use of the tourniquet over four hours may result in the rate of viable limb salvage decreasing by over 50% [15]. Depending on the injury's location, muscle density may be a large factor in the rate of cell degeneration. For example, a leg injury may require more urgent attention as the thigh and calf are composed of a high muscle mass, whereas an arm does not have as high of a muscle density. The situation is similar if dealing with a man or woman, as men naturally have a denser muscle composition than women [16]. Since muscle has the shortest ischemic times (regardless of warm or cold), a quick solution to prevent necrosis will be crucial in preserving its viability.

In order to avoid necrosis, it is important to understand the various types of amputations. Injuries that result in amputation can be a result of three major causes: crushing, avulsion, and laceration. Crush amputations are a result of heavy compressive force being applied to a part of a body. It often results in crush syndrome where the limb that is affected swells, the soft tissues are damaged, and the body starts to dysfunction causing cell death. Depending on the severity, crush syndrome can begin within twenty minutes after an incident or an hour later. If compressive forces prolong for over four hours, the limb, and other organs around it will start to lose function, and after six hours limb necrosis will occur. Even though crush injuries are not common, it is mostly due to natural disasters or an attack of violence. 83% of crush injuries are of the lower extremities and the remaining 17% on the upper extremities.

Avulsion amputations are also referred to as tear amputations. This is when there is a tension force on the limb causing the limb to be stretched and the nerves and tissue to be pulled apart. Tear injuries that lead to amputations are rare and are common in the hand. The most common reason for these injuries is small accidents at the workplace or playing a sport.

Lastly, there are laceration amputations or guillotine amputations where the entire bone, muscle, or limb is cut off. This is commonly seen when the limb is salvageable after trauma or in health issues as seen in highly diabetic patients. This type of amputation is mostly seen in the lower extremity. If the limb is salvageable, the limb is often attached back with polymethylmethacrylate antibiotic (PMMA) bone cement. Laceration amputation can be dangerous as it is very prone to infections. Medical professionals are told to follow the “no touch” rule, where the limb encounters only sterilized tools for examination. A tourniquet is not required for this amputation to control blood flow since the arteries and veins are exposed and can easily be identified to control blood flow. Some causes of laceration amputations are car accidents, war, and dangerous workplaces. These three main types of amputations have different mechanisms and therefore make it difficult to find a generic solution to save limbs for all three amputations.

2.2: Situations

According to the national electronic injury surveillance system database (NEISS), children aged 0-5 years are most likely to get amputations. The second most likely age group is 51–55-year-olds. Males make up 75% of amputation cases. Caucasian males had a higher chance of getting an amputation than any other demographic group worldwide. Even though military officials are more at risk for injuries leading to amputations, it was found that 4-40% of amputation cases were from civilians and 5-15% were from military patients. It is estimated that by 2050, 3.6 million people would be suffering from limb loss and 65.9% of replantation surgeries fail. There are several factors that could affect replantation of severed

limbs including age, blunt trauma, injury location, ischemia, reperfusion injury, and the severity of the injury. Failure could also occur due to bacterial infection, thrombosis, or vascular crisis [17].

An overwhelming 91.5% of traumatic amputations are of the finger. This is followed by the toe (5.4%), hand (1.0%), and the foot (0.7%). The lower leg, lower arm, upper arm, and upper leg were only hbo0.3%, 0.2%, 0.5%, and 0.5% of the cases overall, respectively [18]. Although less common, these major limb amputations are much more likely to cause death and have a much higher impact on the patient, physically and mentally, if they are not able to be reattached.

In the military, there are three main causes of major traumatic amputation: stepping on a buried mine (lower limb injuries), device exploding near the victim (lower limb injuries), and a device exploding while the victim is handling it (upper limb injuries) [19]. The highest occurring mechanism of injury is due to blast (72.8% of total military injury) [20]. Following blast, the remaining military-related injury result from penetration (23.5%), blunt (1.4%), or unknown causes (2.4%) [20]. For civilians, these types of injuries are not always as common due to the lack of combat situation. The leading cause for injury in civilians is penetration (47.7%), followed by blunt (36.4%), unknown (9.4%), blast (4.1%), iatrogenic (illness) (1.3%), and animal bites (1.1%) [20]. Over 50% of their injuries occurred within their own home caused by doors and power tools [18]. Major traumatic amputations are caused by motor vehicle accidents 42.9% of the time, industrial accidents 26.2% of the time, and motorcycle accidents 21.4% of the time. Causes may also be classified as unintentional, such as falls, fire, poisoning, animal contact, and forces of nature. Intentional causes may include self-harm, interpersonal violence, and other conflicts [21]. Of these accidents, over 10% of cases are above the elbow, 17% are above the knee, 19% are below the elbow, and 53.2% are below the knee [20]. This makes major traumatic amputation of the lower extremities more common in both military and civilian demographics.

2.3: Solutions

When a limb is trying to be salvaged, the current solution is to wrap the limb in a bandage soaked in saline solution inside of a bag and placed in ice water. It is recommended to not place the limb directly on ice to prevent frostbite and to not use dry ice as well. A hypothermic solution is most common because ice reduces inflammation, decreases metabolic function and blood flow helping preserve the structure and function of the limb. If the limb is not properly preserved, its chance of reattachment decreases significantly. Cold ice packs compress reduce inflammation by constricting blood vessels and decreasing blood flow to that area. Heat on the other hand expands the muscle and blood vessels which increases inflammation. Ice packs decrease metabolic function by maintaining homeostasis. When an area is introduced to a cold compress, it causes the body to increase the temperature which in turn keeps the body at a regular temperature. There are many other methods out there that attempt to increase the chances of limb salvage. These include changing the temperature to be normothermic, hypothermic, or using alternating hypothermia. This can be done by various methods like sublimation, compressed gas expansion, and chemical cold packs using endothermic reactions. Other alternatives are hyperbaric oxygen treatments and ex vivo perfusion. These options are not currently available for the application of limb salvage but show promise in their associated indicated uses. To understand the various solutions, it is vital to understand how cooling and heat release works.

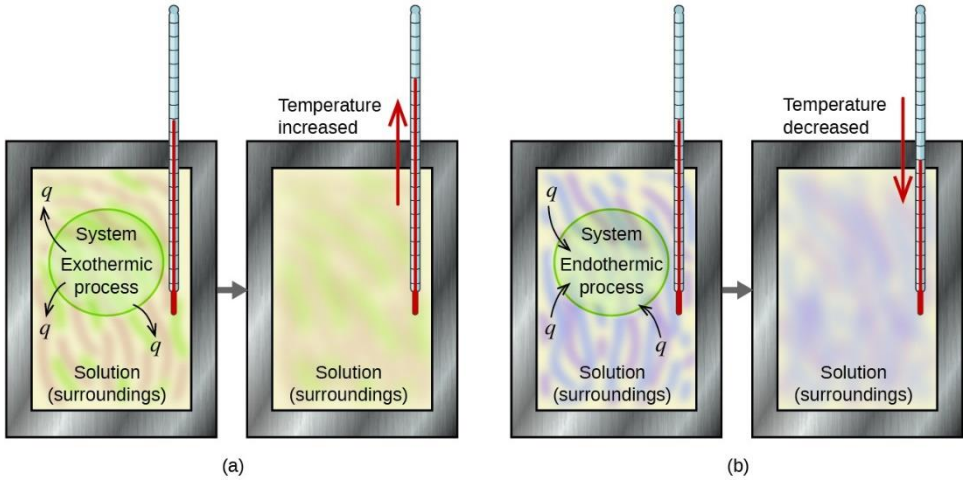


Figure 2 Calorimetry Diagram [22]

To find the amount of heat released, the calorimetry equation can be used. The equation is:

$$Q = C \times m \times \Delta T.$$

Q is the heat of a substance, C is the specific heat of the substance where it represents the amount of energy needed to increase the temperature by one-degree Celsius, m is the mass of the substance, and ΔT is the change in temperature. The units for Q are Joules (J), the units for C, are J/(mol•°C) if pressure is constant (Cp) and J/(g•°C) when heat capacity is given via mass (Cs), the units for m are grams (g), and ΔT is in °C. If Q and ΔT are positive that means heat is flowing away from the surrounding, and if Q and ΔT are negative, heat is flowing towards the surrounding. It is an exothermic process when Q and ΔT are negative and an endothermic process when Q and ΔT are positive. Both Q and ΔT can either be positive or negative at the same time but one cannot be positive while the other is negative and vice versa.

As previously mentioned, there are a variety of ways to achieve this endothermic reaction. The main three ways are sublimation such as dry ice turning from solid to gas, compressed gas expansion such as the systems used in refrigerators, and chemical cold packs such as the ones typically used in sports injuries.

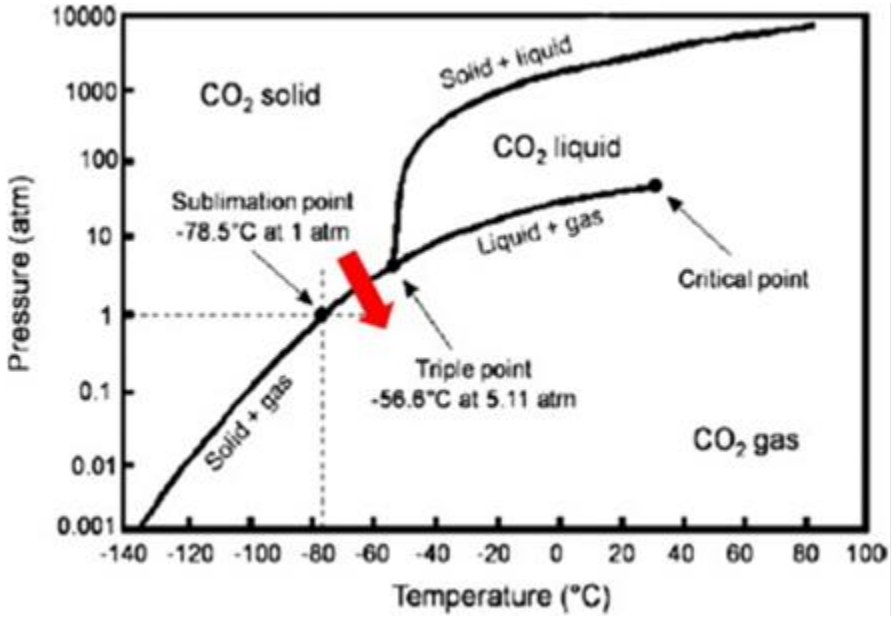


Figure 3 Image Showing a CO_2 Phase Change Diagram [23].

Sublimation is defined as the phase change of a substance directly from a solid to a gas without passing through the liquid phase. CO_2 is a common compound that demonstrates this phenomenon and is more commonly known as dry ice. There is a direct relationship between temperature and pressure when it comes to sublimation, and this is shown in figure 3 below for CO_2. The triple point represents the point where all three phases (solid, liquid, gas) come together. The sublimation point represents the conditions in which sublimation can occur for CO_2. Between these two points, along the sublimation line, is where sublimation will occur if it falls below as the red arrow indicates [23]. Direct contact of dry ice with the skin will increase the damage to the tissue as well as increase the formation of crystals [25]. Moreover, the extremely low temperatures can sometimes cause further complications.

Indirect ways of cooling can help to provide a cool environment for the limbs to be transported and have higher chances of viability for reattachment. By being able to provide cooling in a regulated system, the limb will be ensured to stay in a constant environment that provides the highest benefit. One method of indirect cooling is compressed gas expansion. Compressed gas expansion has many applications outside the body, such as refrigeration, air conditioning, compressed air dusters, and electric generators. All systems accomplish the same goal of cooling in slightly different manners. Oftentimes, a refrigerant (chemical liquid hydrocarbons) is used to achieve cooling, as the liquid is contained within high pressure conditions that result in an equilibrium of gas and liquid [25]. Specifically, air can dusters (and other aerosol products) use 1,1-Difluroethane as the liquid [26]. Since 1,1-Difluroethane has a boiling point of 52.3 degrees Fahrenheit at 760 mmHg (atmospheric pressure) [27]. In compressed air cans, the liquid and gas maintain equilibrium until the nozzle is pressed, opening a valve, and releasing the gas. This release of the gas lowers the pressure placed on the liquid, bringing it to its boiling point, producing more gas until equilibrium is again reached and the valve is closed [28]. Similarly, other air-cooling technology uses other refrigerants to accomplish a similar process.

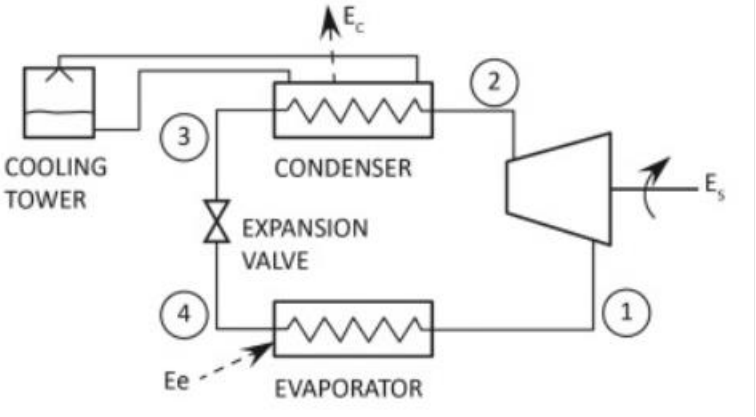


Figure 4 Demonstration of Compression Refrigeration Cycle [29].

The compression refrigeration cycle (seen in Figure 4) is commonly used among most refrigerators and air conditioning systems. Within the cycle, the vaporized refrigerant flows from an evaporator through a compressor. With the aid of electrical input, the evaporated refrigerant is compressed from its pressure at the evaporation stage to the pressure that is required for the liquid to condense while simultaneously increasing the vapor temperature [29]. Remaining at a constant pressure, the resulting heat/vapor of the reaction is then cooled and condensed in a condenser filled typically with cooled air or water. After being condensed, the refrigerant returns to liquid as it approaches the expansion valve [29]. Once at the expansion valve, the refrigerant's pressure is then lowered, causing it to reach its boiling point to release as a gas. As it enters the evaporator, the gas absorbs the heat at a constant temperature and pressure, ensuring that it remains vapor as it repeats the cycle and enters the compressor [29].

Throughout this process, the enthalpy of the reaction can be measured. Enthalpy is defined in thermodynamic systems as energy is changing, denoted by the variable *H* [30].

$$H = U + pV$$

U is defined as the internal energy, *p* being pressure, and *V* the volume [30]. When flowing from the evaporator to the compressor, the energy can be measured by subtracting the enthalpy following the compressor from the enthalpy before entering the compressor [29]. By subtracting the enthalpy following the compressor from the enthalpy of the liquid following the condenser, the heat rejected is calculated [29]. Lastly, to determine the heat absorption by the evaporation process, the enthalpy following evaporation must be subtracted from the enthalpy of the liquid entering the evaporator [29].

Another form of cooling is through the use of chemical cold packs (CCP). CCPs are a common form of cooling when ice is not available. They are typically used in first aid for situations like a sports injury or heat illness and. CCPs are composed of a small plastic bag and a tube inside. When the CCP is squeezed the tube inside breaks causing its components to mix with the water inside the bag. The chemicals are typically ammonium nitrate, calcium ammonium nitrate, or urea [31]. Once one of these chemicals mixes with the water an endothermic reaction occurs. As previously mentioned, an endothermic reaction is a chemical reaction that absorbs heat from the environment. CCPs are helpful at relieving pain caused by injury by numbing the area through means of cooling. Due to the same mechanism of treatment as state-of-the-art, CCPs also help reduce swelling, inflammation, and bleeding. However, there are many drawbacks to CCPs in the scope of traumatic amputations.

The major drawback is that CCPs are not as effective as ice packs. Ice packs can provide a significantly higher enthalpy change over a longer period than CCPs of the same or similar sizes [32].

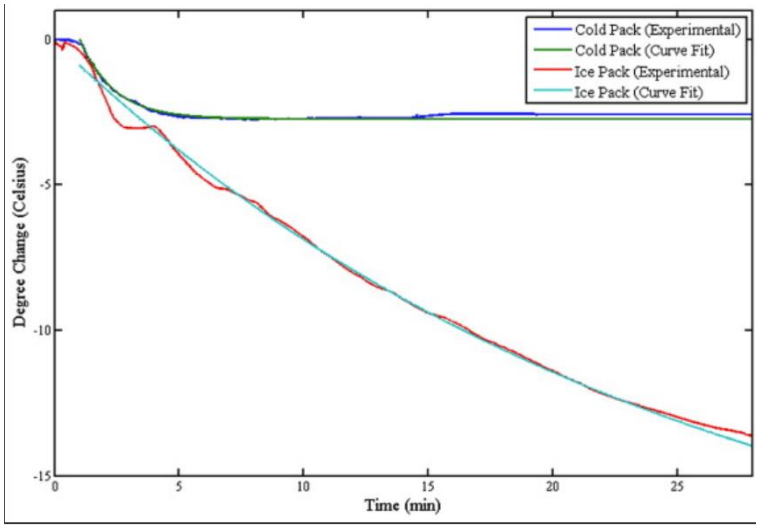


Figure 5 Effectivity of CCPs vs Ice Packs [32]

As seen in Figure 5, over the span of 25 minutes the CCP was able to decrease the temperature by less than 5℃ whereas ice packs were able to lower the temperature by nearly 15℃. The average change in temperature achieved by CCPs and ice packs is 5.245℃ and 19.8℃, respectively [32]. Furthermore, in ambient environments of over 37℃ the CCP would not be able to effectively lower the temperature to a therapeutic range. Due to the larger quantity of tissues present in major traumatic amputations (such as upper arm and upper leg amputations) and limited enthalpy change allowed by CCPs, they would also not be able to cool the whole limb effectively to the therapeutic temperature. Therefore, although helpful in some circumstances, CCPs are not useful in the case of major traumatic amputations.

An alternative form of treatment that does not involve cooling is hyperbaric oxygen treatment (HBOT). It is 100 percent exposure to oxygen with 2-3 times the atmospheric pressure at sea level [33]. The medical applications of this treatment have been studied for some years, but it is still a relatively new treatment method. One common application is to treat reperfusion injury, which is when the cells start dying after the blood flow has been restored in ischemic tissue. Another use is to treat carbon monoxide poisoning as it significantly decreases the amount of carboxyhemoglobin - its half-life goes from 4 hours to 20 minutes - from regular air [33]. HBOT is also being explored as a treatment for post-operation purposes, such as for reattached severed limbs. Mendy Hatibie Oley and colleagues conducted a study on 2 separate patients with severed hands where HBOT was used post-operation to help the healing process. In both cases, the patients were able to gain at least some functioning of the hand back, regardless of how the limb was transported [34]. HBOT is also administered in certain segments of time for multiple treatments. For this study, both patients were given 3 days of HBOT at 90-minute intervals [34]. The level of perfusion of oxygen using HBOT is also not clear, but it is speculated that it is effective for soft tissues in crush injuries and acute traumatic ischemia cases [35].

HBOT is a useful treatment, but it is not perfect. Middle ear barotrauma is one of the most common side effects of HBOT and results in feelings of pressure, pain, and discomfort in the middle ear [36]. It can also lead to more serious medical complications if left untreated such as ear infections, hearing loss, or vertigo. Sinus barotrauma is another side effect that can occur and has similar effects to the middle ear barotrauma. There is pressure in the paranasal sinuses and the increase in pressure from hyperbaric oxygen therapy treatments would induce more facial pain as well as congestion [36]. HBOT can also cause central nervous system oxygen toxicity due to exposure of high amounts of oxygen. Although it isn't very common, and the pathology is not completely known, CNS oxygen toxicity is speculated to be due to be due to nitric oxide-forming rite [36]. This oxygen toxicity in very rare cases can cause a seizure due to the higher levels of CO_2 being retained. Lastly, myopia is another common side effect of receiving hyperbaric oxygen therapy. This is usually only seen in multiple recurring treatments

and is thought to be because of damage to crystalline structures in the lens proteins [36]. Most side effects of HBOT are not very common and usually only happen with continuous and repetitive exposure.

In the world of organ transplantation, normothermic ex vivo perfusion is used as a way of moving organs from one location to another without the risks of major tissue decomposition. At a high level, ex vivo perfusion works by keeping organs at a normothermic temperature and oxygenates the tissue by continuously pumping blood through them [37]. These machines are currently used to reduce the time the organ spends in ischemic conditions. With normothermic ex vivo perfusion, the organs can remain viable outside of the body for longer [37]. The problem with this technology, however, is its inaccessibility to most departments and very costly. The average cost of an organ transplant using ex vivo perfusion is $87,714-$136,000 [38].

Many studies have been done to research how this technology can be implemented for traumatic amputation. Diving in deeper to the technology, the device is made up of four main components:

1. a perfusate reservoir,
2. a pump,
3. an oxygenator,
4. and sensors.

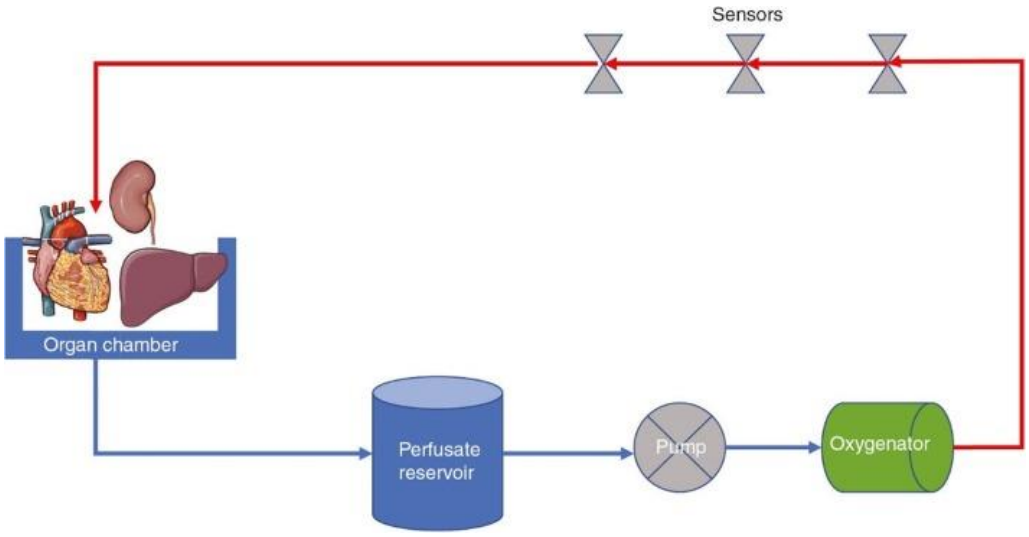


Figure 6 Ex Vivo Perfusion System [39]

The system for ex vivo perfusion can be seen in Figure 6. The perfusate reservoir typically contains a solution of both plasma and hemoglobin (the components typically found in blood) [39]. The blood is then pumped through an oxygenator and sensors are used to monitor the blood. The blood is then pumped through the limb and back out through the perfusate reservoir. This process can be repeated for eight to twelve hours in cases of organs like the heart and lungs [39]. However, studies testing the viability of 64 porcine forearms perfused for up to 12 hours showed that replantation surgery was feasible without an increased risk for ischemia and reperfusion injuries (IRI) [40]. Another study tested near-normothermic ex vivo perfusion in five human arms with results showing the potential of tissue viability [41].

A significant drawback of this method is it hasn't been tested clinically to show the ability for the limb to be reimplantable after this method. Another drawback is these devices are quite large and would be even larger if being used for the application of severed limbs. Due to this it is nearly impossible for these devices to be easily portable. Furthermore, they are also extremely expensive. This limits the accessibility of these devices drastically, as can be seen in how limited the access is to ex vivo perfusion systems for organs.

2.4: Common causes of replantation failure

There are three major types of failure in replantation surgery: total failure, poor function, and no function [42]. Total failure is when the surgeon is unable to successfully attach the limb due to poor decisions or techniques used. It also can be associated with problems relating to the coagulability of the blood, but this is rare. Poor function is when there is anatomical restoration but due to ischemia, joint dysfunction, poor nerve coaptation, or infection the functionality of the limb is impacted. This typically results in significant scarring. No function is when the patient is unable to move or has sensation of the limb [42].

Typically, failure can be avoided by reducing procedure times and thus ischemia time using methods such as rapid bone fixation and appropriate vascular clamps [42]. Proper evaluation of the tissue is also crucial to save viable tissue and reduce the risk of more complications in the future. Postoperative care can also assist in reducing functionality failure types by monitoring patients as well as incorporating physiotherapy [42]. However, even if everything is done correctly and all risk reducing measures are put in place, there are still situations that increase the risk of replantation failure.

There are different types of situations that impact the risk of replantation failure: mechanism of injury, time after injury, and patient demographic. The types of injuries that increase the risk of replantation failure are major crush, avulsion injury (when skin, tissue, or a body part is torn forcefully away) such as nerve avulsion, or multiple levels of injury [43]. Time after injury is split into two categories: warm ischemia and cold ischemia. Amputated limb viability greatly decreases with a warm ischemia time of more than 5 hours or a cold ischemia time of more than 12 hours [43]. The patient demographic that most greatly increases the risk of replantation failure is age. The older the patient is the higher the risk of replantation surgery being successful. A study on the risk factors for failure of an upper extremity replantation found that 56.2% of patients over 50 had replantation failures compared to only 39.6% of patients under 50 had replantation failures [44].

Another impact to replantation failure is reperfusion injury. This is the increase in cell death cause by the restorations of blood flow to ischemic tissue.

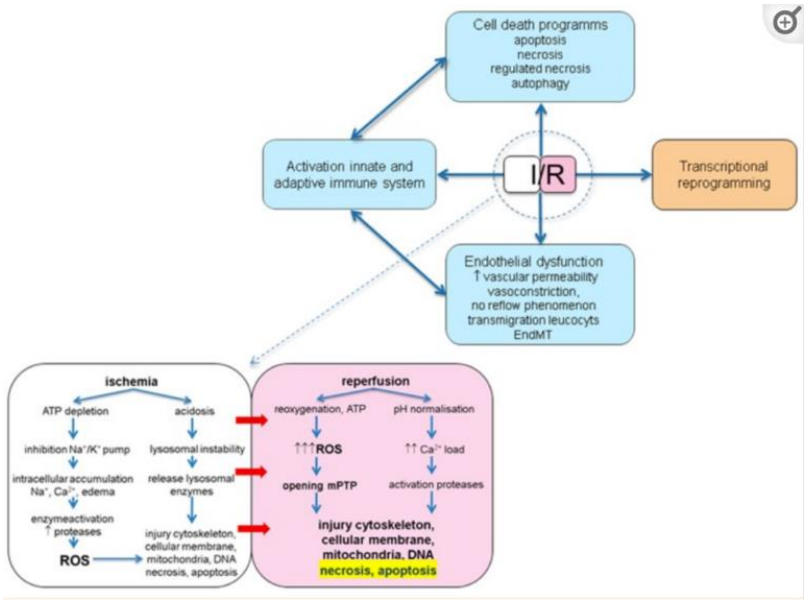


Figure 7 Mechanism of Reperfusion Injury [45]

Figure 7 demonstrates the consequences of ischemia and reperfusion. When reperfusion occurs, the cells are reoxygenated. This reoxygenation allows the pH to return to a normal state. The calcium in the blood also causes a sudden increase in calcium ions in blood. This in turn activates calpains (calcium-activated proteases) which ultimately leads to cell death through apoptosis [45].
. Reperfusion also triggers an increased production of reactive oxygen species (ROS) produced in the cell mitochondria. This is dangerous because ROS can cause damage to the membranes, cytoskeleton, and DNA of cells. ROS can also disrupt the creation of adenosine triphosphate (ATP), an energy storage molecule, made in the mitochondria [46]. This will cause the mPTP induction and the release of substances that further increase the risk of cell death through apoptosis or necrosis [45]. Reperfusion injury can be reduced by limiting the oxidative stress and the creation of ROS in the mitochondria of the cell.

The ideal amputation for replantation surgery is on a young patient with a “guillotine amputation” or laceration amputation [12]. This is because there is significantly less damage to the surrounding tissues and vasculature compared to a crush or avulsion injury. Also, a young patient has a higher chance of replantation success.

Chapter 3: Project Strategy

Based on speaking with the project advisors, the team obtained an official client statement. This statement includes what the design must accomplish within specific parameters. After understanding the scope of the project and the main applications where the device would be applied, the team created a detailed list of design requirements that were essential to accomplishing the objective of limb salvage. These requirements mainly consisted of time, portability, and ease of use. Standard regulations including ISO, FDA, ATSM, etc. were also considered to ensure the device would be a manufacturable product that met safety requirements. After working with the advisors and based off research and preliminary work, a revised client statement was produced to detail a more accurate depiction of the project. Lastly, the team agreed upon a management approach that would ensure the timely completion of the project and specific goals.

3.1: Initial Client Statement

The team is looking to create a device for adult major traumatic amputation patients that is easily transportable, can preserve the limb for a minimum of six hours in ambient temperatures of –30°C to 38°C and will improve reattachment success in replantation surgery.

The design must:

- Be easy to use by medical professionals
- Be biocompatible
- Be cost effective
- Have easily accessible materials
- Meet necessary standards (FDA, ISO, etc.)

3.2: Technical Design Requirements

Based on the initial client statement and project objectives, the team determined the constraints. These constraints limit the design parameters to meet the desired functionality based on situation, geographical location, and specifications. To narrow the scope of the project while covering most cases, the project will focus on a few main categories. Preliminary assumptions were developed after ruling that adults suffered most major limb injury. The first assumption the team made is that this device will be used primarily in adults. Although children experience amputations, it is often of the fingers and toes rather than major appendages. The device will also be used in situations where trained medical professionals, such as EMT, nurses, medics, or doctors are main contributors in the application.

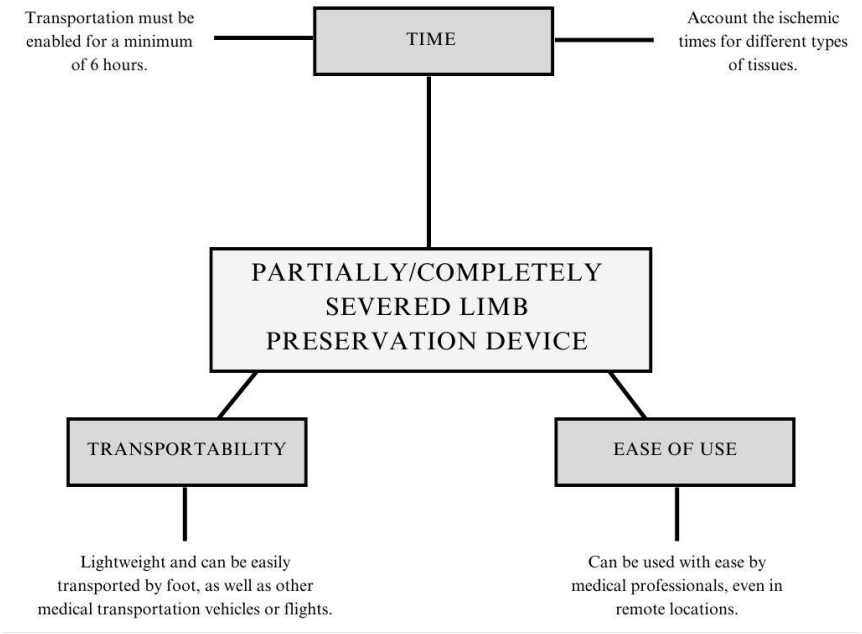


Figure 8 Concept Map of Project Constraints

When preserving limbs that have been partially or completely severed, the main goal is to overall reduce metabolic rates and ischemic times. When the limb is not completely attached to a constant blood supply the tissue in the appendage begins to degrade as metabolic activity is still mobile within the limb. Due to the severity and nature of the injuries the device works to preserve, there are major constraints that must be considered. When designing a device for this situation, the team mainly considered and brainstormed around 3 major constraints: time, transportability, and ease of use, as demonstrated by Figure 8.

Since time is a major component of severe injury resulting in partially or completely severed limbs, this was deemed to be one of our focus areas. Due to recommended ischemic times of specific tissues such as muscle tissue, depending on if it is kept at room temperature or chilled for no longer than 4-8 hours, the device would need to ensure that the limb is kept stable. The location of where the injury happens is also a crucial factor. For example, if the injury were to happen in a remote location, like a desert or the mountains, it will take time to get to a location where reattachment surgery can take place. Therefore, the device should be able to operate in at least 6 hours of transportation if necessary. Circumstances like these may be especially common in military situations where soldiers are stationed at remote locations in difficult terrain. The goal is to preserve the limb long enough to increase successful reattachment rates by reducing the ischemic time and minimizing metabolism in the limb.

Depending on the geographic location of the injury, the person may need to be rescued. This may occur by foot, vehicle, or aircraft. Some situations may require the person to be attended to by a medic on foot. For example, if the person is stuck in an avalanche situation, a helicopter may fly in, but a medic will ultimately need to travel some distance by foot to reach the injured person. In addition to foot travel, a helicopter may be used in extreme situations where vehicles are not able to reach, or time is critical. Lastly, medical vehicles are the most common manner of rescue, for example, in cases of car accidents, at home injuries, etc. Given the versatility of circumstances the device may need to be transported in, it must be small and light enough to be carried and stored in locations accessible to be carried. In addition to being carried, a separate device of slightly greater size may be considered as a stationary solution once the injured person has reached a medical vehicle or aircraft. This device may be a little greater in size and weight as it will remain in its position and unmoved. The limitation with a device like this would be ensuring that it fits in the helicopter, ambulance, etc. It also needs to be properly powered through materials already present in those modes of transportation.

Once he injured person is in the care of a medical professional, it is important that the device is easy to understand and be used by the medical professional in a timely manner. Since the shortest recommended ischemic time is 4 hours for muscle, time is crucial. Eliminating any unnecessary or complicated steps will help to avoid wasting time. The design should also be intuitive to trained professionals. The device should be easily understood by EMT, medic, and other healthcare personnel. If there are any unintuitive elements, training for use of the device should be provided. The core element of the device should be apparent, any secondary processes should be readily prepared.

In addition to the main constraints, the design's material properties should be considered. For a device that will be used in a variety of circumstances, the team needs to consider a broad range of material properties that will go into the design. First, the materials used in the design should be able to withstand both high and low temperatures, due to varying geographical locations it may be used in. Expanding on this, the materials should not become brittle in cooler temperatures and should not deform in warmer temperatures. Overall, the design should withstand a temperature range of –30 to 38°C. Alongside temperature, the material should withstand changes in pressure. The structure of the material should be stable under varying ranges of pressure. If the device includes an internal pressure, the casing material should ensure that the pressure within can be regulated and maintain its desired temperature and pressure. The material must also be biocompatible. The body should respond positively to the device and not complicate the process or induce further injury. It should also be in accordance with ISO regulations, as described in section 3.3. The design material should be cost-effective and be easily accessible. Materials should undergo different types of testing including tensile, inflation, stress, etc. to ensure its rigidity for the intended application.

3.3: Standard Design Requirements

When considering the essentials for design requirements in medical devices, there are multiple factors that would affect the ability to impose the necessary reactions with the body. There are many different organizations that assist in setting these standards. The most common are International Organization for Standardization (ISO), American Society for Testing and Materials (ASTM), Institute of Electrical and Electronics Engineers (IEEE), International Electrotechnical Commission (IEC), and the FDA's Title 21 Code of Federal Regulations.

It is important when designing a medical device to have an appropriate quality management system (QMS). This is a set of documents that include the specific design, production, and implementation methods of the device (ISO.org). The standard followed for the creation of a QMS is typically ISO 13485 [47]. The FDA is currently working on changing the regulations in the U.S. to follow more closely with this standard.

Sterilization is an important aspect of this project as the device would be used on open wounds, so sterility is highly essential. This would include having a sterile environment during production as well as packaging. ISO standards for sterilization are found in ISO 19930 and outline the allowable levels of microorganisms that exist in a healthcare product when distributed [48]. Biocompatibility is a primary example of this and is the device's effect on the human body. The materials used must not have any toxic or negative long term or short-term effects on the patient. In the case of this project, it is a more short-term use, but the materials used to create the device must be researched to ensure it is suitable for the body parts and bodily fluids it will contact. ISO 21726 defines the allowable toxicity levels for the materials and device overall [49]. Furthermore, according to FDA's "Guidance for Biocompatibility Testing of Medical Devices" it is recommended to also use ISO 10993-4 and ASTM F756 to test for direct and indirect hemolysis (destruction of red blood cells), ISO 10993-5 to test for if extracts from polymer material used is cytotoxic, and ISO 10993-12 to prepare samples for the testing types included in the ISO-10993 standard [50]. In terms of medical products that handle blood it is also important to follow ISO/TC 76 which details the standards for medical equipment that handles blood: equipment that deals with transfusion, infusion, injection, and processing of blood.

If the device has electrical components, it is important to follow the FDA's "Guidance for Basic Safety and Essential Performance of Medical Electrical Equipment, Medical Electrical Systems, and Laboratory Medical Equipment". Some required standards listed in this document are within the scope of the device. This includes ANSI/AAMI ES60601-1 and IEC or IEC 61010-1 which illustrate the basic requirements for safety and performance in laboratory, IEC 60601 which goes over the requirements for medical electrical equipment and systems usability in the emergency medical services (EMS) environment, and ISO 80601-2-69 which explains the basic safety and performance requirements of oxygen concentration equipment.

When it comes to testing, often medical applications first use animal testing with similar anatomy. To remain within ethical and production guidelines, ISO 10993 is used to determine the use of such animals [50]. It is important to note that the FDA does not fully recognize this standard. Thus, to apply with FDA standards some modifications. These modifications can be found in FDA's "Guidance for use of International Standard ISO-10993" but they include the addition of endpoints for chronic toxicity, carcinogenicity, reproductive/developmental toxicity, and degradation. The guideline also highlights the categorization of the different types of exposure and the corresponding assessment. For the scope of this project, all ethical guidelines will be followed, and no live animal testing will be conducted during the duration of this project. All standards will be carefully considered when choosing materials and their impacts for this design.

3.4: Revised Client Statement

Based on preliminary research, project strategizing, and discussion with our advisors, the team revised the initial client statement to fully capture what the device must accomplish. The statement reads:

The team will create a lightweight and portable device used by medical professionals to readily treat adult patients suffering from recent major traumatic amputation injuries. The device must preserve tissue by cooling to a target internal temperature of about 16 to 18 degrees Celsius for a minimum of six hours during transportation to medical centers, increasing the limb's viability. The device must also work in wide ranges of temperatures given the varying scenarios these injuries may occur.

3.5: Management Approach

To best manage project work, the team has developed organization methods to ensure a timeline and deliverable requirements are met in a timely manner. The team used Microsoft Excel and Planner to create our work breakdown structure, Gantt chart, and planner (based on Gantt chart). The Gantt chart outlines each term's major deliverables with appropriate breakdowns on how they will be achieved throughout the term. The team plans to outline each term the week before the previous term ends. This ensures each term is properly planned according to the previous term's progress. The chart consists of the assignment, task owner (if any), start date, due date, duration, and progress. Based on the start and deadlines dates, the blocked section to the right is filled in with the appropriate duration. An example of the Gantt chart can be found in Appendix A. The work breakdown structure (WBS) was created by making columns of step of the process we deemed as important. Under each column were specifics of what needed to be done to accomplish each main category. For example, as demonstrated in Appendix A, the "project prep" phase was followed by the tasks of solution vision, brainstorming, initial and in-depth research. A similar pattern was followed for planning, development, final prep, and presentation phases. Lastly, the team created a planner to organize bigger tasks shown on the Gantt chart and smaller tasks that may occur weekly. The tasks are organized into "buckets" as follows: initiating, planning, executing, monitoring and controlling, closing, and complete. The task is assigned a title and its appropriate details

as well as a due date. As a task phases out of initiation, it then moves to planning where the beginning steps are taken to prepare for the task. For example, if the task is “B Term Goals”, the team is in the process of filling out the Gantt chart and deciding on deadlines and other details. As the task is passed to the executing bucket, this denotes that the task is actively being worked on. From here, it can be moved to monitoring and controlling or closing depending on the status of the assignment. If the assignment needs more work throughout the term, the team will place it in the monitoring and controlling bucket. If the task is approaching its last steps, it will be moved to the closing bucket before being marked complete. This process can be seen in Appendix A.

Chapter 4: Design Process

After much preparation, including research and conversations, the team moved forward in with the design process. The initial step was producing the needs analysis based on the client statement. The need was broken up into the problem, population, and outcome, therefore resulting in a needs statement. Once the general need was detailed, the team then began brainstorming the main objectives of the device and ranked them against each other using a pairwise analysis. This allowed for identification of the most important objectives in creating a device design. An overall concept map was developed to showcase the research and knowledge of the team when moving forward to creating design ideas. Four main designs were developed by the team, three of which resulted in alternatives to the final design.

4.1 Needs Analysis

The need of the client is always the most important objective to meet in any design project. Ensuring the need is defined properly will aid in successful client satisfaction. To understand the scope of the client's need, the problem, population, and desired outcome(s) can be broken down. As seen in Table 1, the problem of the project is defined to explain that what is currently being done is not sufficient to overcome the need. Next, the audience must be considered. Based on the assumptions the team made for the project, the audience was defined as adults who suffer from major limb injuries resulting in severance. Lastly, the desired outcome of what the client intends the design to accomplish, in this case, decreasing tissue necrosis.

Table 1 Scope of the need statement broken up into 3 main sectors.

Problem	Current solutions (ice baths) are not effective enough at minimization of tissue necrosis for successful reattachment
Population	Adult patients with severe appendage injuries that result in partially or fully severed major limbs (legs and arms)
Outcome	Producing a device that reduces tissue necrosis in ischemic limbs for at least six hours (in transport) to enable higher success rates in reattachment

Based on the table above, the team formulated a needs statement that captures the problem, population, and outcome into a short blurb:

"A way to address current ineffective methods for minimizing tissue necrosis in adult patients with partially or fully severed major limbs. The anticipated outcome is to produce a device that can improve success rates of replantation surgery in adult patients with severe appendage injuries for at least six hours in freezing and desert temperatures."

After understanding the need of the client, design specifications were determined while also considering the objectives and constraints of the project. Desirable characteristics of a design were evaluated using a pairwise comparison matrix as seen in Table 2. The design matrix consisted of seven objectives the team deemed necessary for a design. The seven objectives were placed in the top row and first column so they could be ranked against each other to better gauge which were more relevant properties. The characteristic earned a score of 1 if the item in the first column was "much better" (in terms of importance) than the item in the top row. A score of 0.5 meant the item was "better than" that in

the top row, a 0 if they were seen to be equally important, a -0.5 meant “worse than”, and a -1 was seen to be “much worse”.

Table 2 Pairwise Comparison Matrix

Objective	Preservation time	Size/ weight	Ambient OP Conditions	Time to Cool	Cost	Additional Training	Universal use in all conditions	Total
Preservation Time	×	1	1	0	1	0	0	**3**
Size/weight	-1	×	0	-1	1	-0.5	-1	**-2.5**
Ambient OP Condition	-1	0	×	-1	1	1	0	**0**
Time to cool	0	1	1	×	1	1	-1	**3**
Cost	-1	-1	-1	-1	×	-1	-1	**-6**
Additional training	0	0.5	-1	-1	1	×	-1	**-1.5**
Universal use in all conditions	0	1	0	1	1	1	×	4

Based on the pairwise comparison analysis completed above, the preservation time, time to cool, and universal use were the top three design objectives to accomplish. Following, ambient operating conditions, additional training, and size/weight, were objectives that were important to the device, yet not the primary objectives. Overall, cost was seen to be the least crucial factor in the design. Although cost will still be considered, the team will not let it defer the design from being the best possible outcome. Based on these specifications, multiple designs were developed to fit.

4.2 Design Process Stages and Studies

Before preparing designs, the team completed preliminary analysis to fully understand the problem and what we were trying to solve. This allowed the team to develop the full picture surrounding the pathology of amputation, possible solutions, situations in which it may occur, and potential ways of testing. In order to better organize the team’s ideas, a concept map was developed. A concept map

allowed the team to visually categorize our ideas and convey very basic information to supply a basis for further investigation and research. As seen in Figure 9, the concept map starts with the central focus and goal of limb salvage (in major amputation circumstances). It then branches out to address the four main points as previously mentioned. Further branching off each of the four main points are specific areas of research the team would further conduct to find the best possible solution.

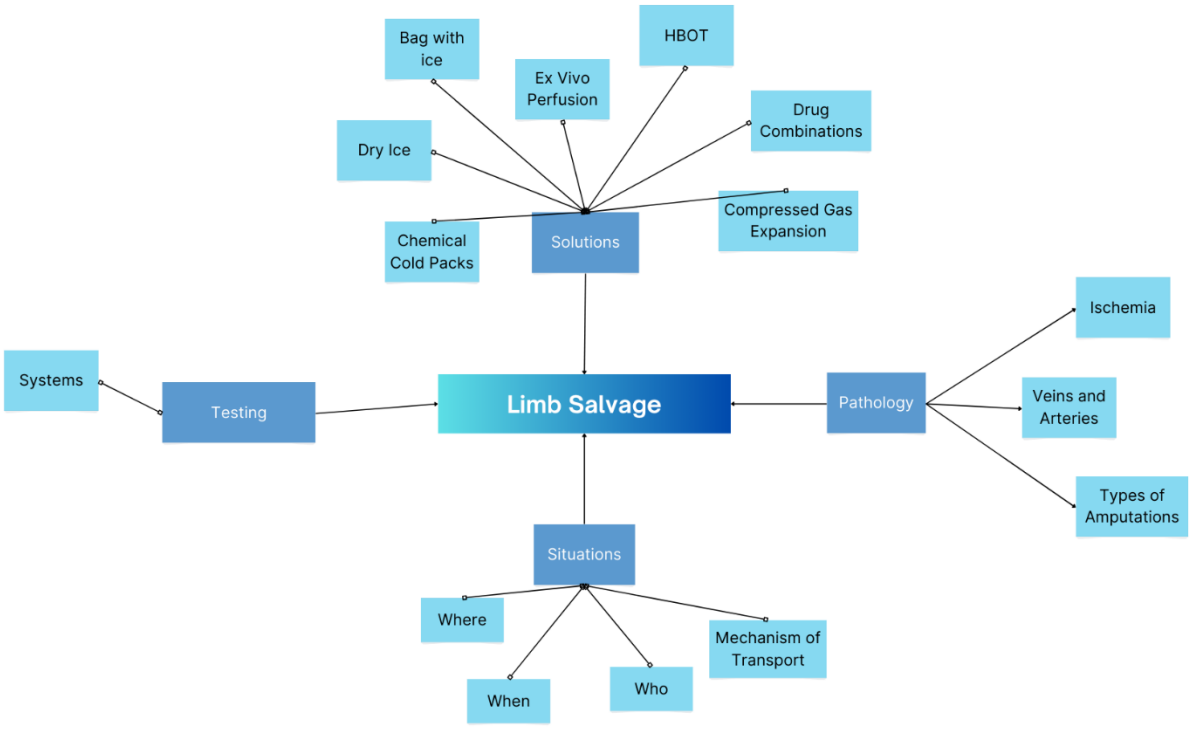


Figure 9 Overall Project Concept Map

Beginning with pathology, the team deemed ischemia, veins and arteries, and types of amputations as three points of focus to better understand the anatomy behind an amputation and how a limb reacts in specific conditions following amputation. This would allow the team to consider the realistic conditions of the limb when designing a device for transport and preservation.

Secondarily, the team considered all possible situations in which an injury that resulted in severe amputation may occur. The team considered when it may happen, where it may occur, who may be involved, and how often does it occur. The team ultimately wanted to understand the demographic of people who encounter severe amputation and the setting of where and how it occurred. This information will allow the team to full understand the scope of the injury to better design a device that fits the majority of the cases.

Lastly, the team thought of all possible solutions and testing of applications to base the device design around. Based on previously mentioned research in Chapter 1, the team brainstormed possible solutions to address the pathologic necessities to properly preserve the limb for viable reattachment. Solutions included the following:

- Chemical cold packs (CCPs)
- Dry ice
- Bag with ice
- Ex-vivo perfusion
- Hyperbaric oxygen therapy (HBOT)
- Drug combinations
- Compressed gas expansion

All possible solutions above were considered by the team and advisors to rule out unrealistic solutions for the application and which to investigate. Based on conversation, the team proceeded to investigate variations of dry ice, ex-vivo perfusion, and drug combinations. Ultimately settling on the following four design ideas:

- Reattachment via cannulation: simulating blood flow of a healthy limb using cannulas
- Internal perfusion cooling: cooling the limb internally via a cooled liquid perfusate
- External cooling via dry ice: cooling the limb from outside in with use of dry ice sublimation
- Ex-vivo perfusion: simulating normal conditions of a healthy limb using external pumps

An in-depth analysis was performed, as later mentioned in this chapter.

4.3 Alternative Designs

Three alternative designs for prolonged limb preservation were researched: cannulation, perfusate cooling, and ex vivo perfusion. Cannulations sought to replicate natural blood flow conditions by reattaching the amputated limb to the body's circulatory system, ultimately preventing ischemia by continuously delivering oxygen and nutrients to the amputated limb tissue. In contrast, the perfusate cooling method involves perfusing cooled saline through the circulatory system of the amputated limb, effectively cooling the limb from the inside out. Finally, the ex vivo perfusion strategy involves a process like extracorporeal membrane oxygenation (ECMO). This system also involves cannulation of the limb's blood vessels to pump oxygenated blood through the limb without reconnecting it to the rest of the body. These innovative approaches offer advantages as well as many challenges in the design of a device that can increase limb viability and preservation capabilities.

4.3.1 Cannulation

As an alternative solution to cooling, cannulation of the limb's blood vessels would mimic typical blood flow conditions in the limb. When the limb is fully or partially severed, much, if not all, of the blood supply is cut off to the injured portion of the limb. Compared to current state of the art, the design would not require any cooling technology to execute. Cannulation would allow for blood from the undamaged portion of the limb/body to flow to the injured portion of the limb, overall restoring blood flow to the limb, allowing for the tissue to be oxygenated and receive the nutrients the blood typically delivers.

The device would consist of three major components: four cannulas, two extension sets, and a one-liter bag of perfusate solution. Two of the four cannulas would be applied to a vein and artery in the

undamaged portion of the limb (proximal to the amputation site), whereas the remaining two would be attached in the same manner on the damaged limb portion (distal to amputation site). The two extension sets would be used to connect the venous cannulas and the arterial connections. Lastly, the perfusate solution would be comprised of a prophylactic antibiotic (consider cefazolin, vancomycin, gentamicin), saline, and an anticoagulant (heparin) to ensure no blood clots while using the device.

The procedure of use would begin by applying two tourniquets to the injury site: one above damaged tissue and one below. Before attaching any of the cannulation materials to the body, they must be flushed with the perfusate to ensure air has been expelled from the area. The EMS personnel would then cannulate a vein and artery in a section of the undamaged tissue, distal to the amputation site. These connections would be flooded into the limb with the perfusate solution to ensure proper flow and lack of air bubbles. Cannulation would occur in the same manner as described previously to the undamaged tissue proximal to the amputation site. The two extension sets would then be connecting artery to artery and vein to vein, resupplying the damaged/unsupplied portion of the limb with blood flow. The flow would then be supplemented with the perfusate/antibiotic/anticoagulant solution to act as a volume filler and prevent further internal infection and clotting.

Through the identification and analysis of user needs and design capabilities, appropriate design specifications were tailored for a device utilizing cannulation. Key design specifications identified the duration of transport, the ambient conditions the device must operate at, the average limb size the device can maintain viability, as well as the standards for cutting off circulation of blood flow with the tourniquets. Specific values for these design specifications can be seen in Table 3.

Table 3 Design specifications and specific values for cannulation design

Design Specification	Specific Value
Target transport time	6 hours
Ambient operating conditions	16-38 degrees Celsius
Average Limb Size	12 kg
Tourniquet Application	Must not cut flow to cannula
Maximum Volume	43 x 30 x 20 (cm)
Maximum Weight	16.5 kg

The device was also designed with the idea that it would be small, compact, and portable, therefore fitting within the size of an average backpack.

After much consideration, the group consulted EMS personnel via interview to get a better understanding of what these medical personnel are capable of. When speaking with an experienced EMT, they mentioned that it was regular to cannulate different veins and arteries in their everyday work. When educated on the design and execution of our proposed device, they stressed the importance of additional training to properly inform personnel on the device's purposes and applications. Based on these conversations, the team created a table of pros and cons, as seen in Table 4.

Table 4 Pros and Cons of Cannulation Design

Pros	Cons
• No electricity/electrical components • Effective at high and low temperatures • Minimal ischemic time • Fully effective with proper attachment	• Must cannulate veins and arteries which would require additional training to personnel using the device

• Requires little to no maintenance once attached • Simple/compact design • Allows for long transport times • Does not require temperature regulation	• Common typical risks associated with cannulation apply • Arteries would not be able to cannulate on site unless it is done in the distal part of limb

Further consultation with plastic surgeon, Dr. Giorgio Giatsidis, brought to our attention the fact that although this design was clever, it may not be as viable as originally imagined. After further discussion, Dr. Giatsidis expressed the difficulty and rare occasion where arterial and venous cannulation would be viable in this application. This is due to the depth of the main arterial and venous systems, as the superficial circulatory system is not sufficient to cannulate given the magnitude of the injury and necessary blood flow. It was expressed that this is a very tedious and articulate procedure that would be very difficult for EMS or other medical personnel to accomplish especially given the environments with device would be applied in.

4.3.2 Perfusate

A design using perfusion cooling was considered for the purpose of simplifying the device and decreasing the time it takes to cool. The goal was to create a device with minimal parts that used resources commonly available among medical personnel. The perfusion cooling would require significantly less materials and would use the anatomy of a human limb as an advantage. This could also be used in either a partially or fully severed limb.

The internal perfusion cooling design idea includes two2annulas, two extension sets, nine-liters total of a perfusate solution, a compressed liquid CO_2 tank, and a waste bag. The overall setup of this design comprises a tourniquet placed directly above the severed area as well as directly below. A warmed solution consisting of an antibiotic, some saline, and an anticoagulant is connected venously and attached to a waste bag, and a cooled solution is connected arterially both distal to the amputation site. The antibiotic in the solution would be cefazolin, vancomycin, or gentamicin, while the anticoagulant could be a heparin solution. Lastly, the cooled solution is connected to an IV bag with a cooling method attached to the bag. The cooling method for the bag could be a liquid CO_2 that would cool the IV bag externally. The idea behind this design is minimal equipment and the ability to cool the limb internally via cannulated arteries and veins in the limb.

The procedure for this device begins with applying the tourniquets directly above and below the amputation site as previously mentioned. Following this, the cooling method in the IV bag should be activated to begin the cooling process of the perfusate solution. Next, the user should flush the tubing with the perfusate solution to ensure a clean and working system. As soon as this is done, the cannulation should be done for both the vein and the artery distal to the amputation. The user should select a section with undamaged tissue and easy access to the area. The extension set would then be attached from the cannulated artery to the IV bag with the cooled perfusate solution. The extension set should then be attached to the waste bag on the venous connection. Once the setup is complete, release the clamp to allow the perfusate to flow through the limb. When necessary, replace the empty IV bag with a new one.

The design specifications for the perfusate method are listed in Table 5 and outline the general constraints that were considered when outlining the device. These considerations were selected based off calculations performed as well as extensive background research on applicable locations and situations

where a limb would be traumatically amputated. This could include remote locations in varying extreme weather conditions such as a desert or a tundra.

Table 5 Design specifications and specific values for perfusate design.

Design Specification	Specific Value
Target transport time	6 hours
Ambient operating conditions	16-38 degrees Celsius
Average Limb Size	12 kg
Maximum Volume	43 x 30 x 20 (cm)
Maximum Weight	16.5 kg

To ensure that the limb is cooled to the target temperature, dry ice was found to be a reasonable choice. Preliminary calculations were done, and an experiment was conducted. Using the following equation:

$$m_{H_2O} \cdot C_{H_2O} \cdot \Delta T_{goal-environment} = m_{CO_2} \cdot C_{CO_2}$$

Using the equation above, it was found that about 15.2 lbs. of liquid CO2 are necessary to cool an average sized limb of about 12 kg. The experiment performed was used to assess the time it takes to cool the limb with cooled perfusate compared to traditional peripheral cooling. One part of the setup included an IV bag filled with ice water connected to tubing inserted down into a two-liter bottle filled with water for cooling via perfusate. The tubing then extended down simulating the arterial connection and then exited the tube out the side about 75% of the way up for the venous return. The second part of the experiment consisted of room temperature water inside a water bottle placed in a Ziploc bag filled with ice to simulate cooling peripherally. As a control, a water bottle was filled with room temperature water. Refer to Figure 10 for a simple drawing of the setups.

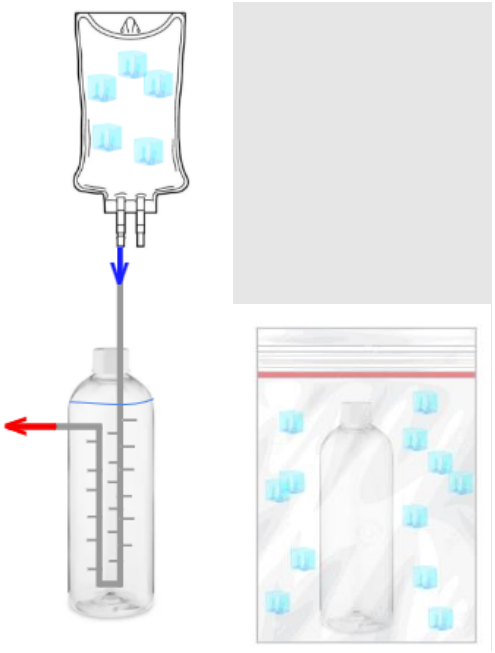


Figure 10 Diagram on the left depicts the perfusate cooling and the image on the right shows the peripheral cooling.

The temperature of each of these items was taken every two minutes. The results were that the peripheral cooling with the ice bag took 42 minutes to cool to the target temperature of 16 °C shown in figure x, while the system with internal cooling took one minute and 45 seconds to cool to the target depicted in Figure 11.

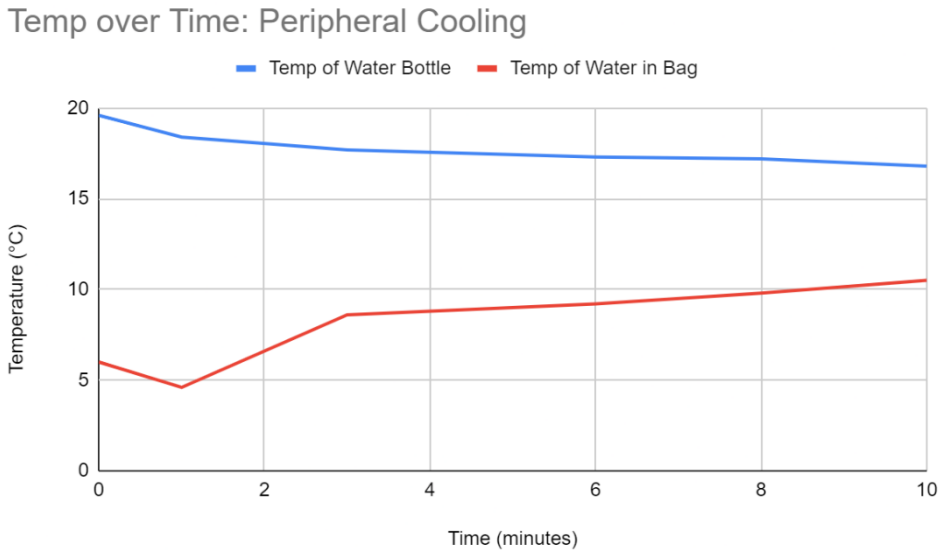


Figure 11 Table depicts time versus temperature of peripheral cooling experiment.

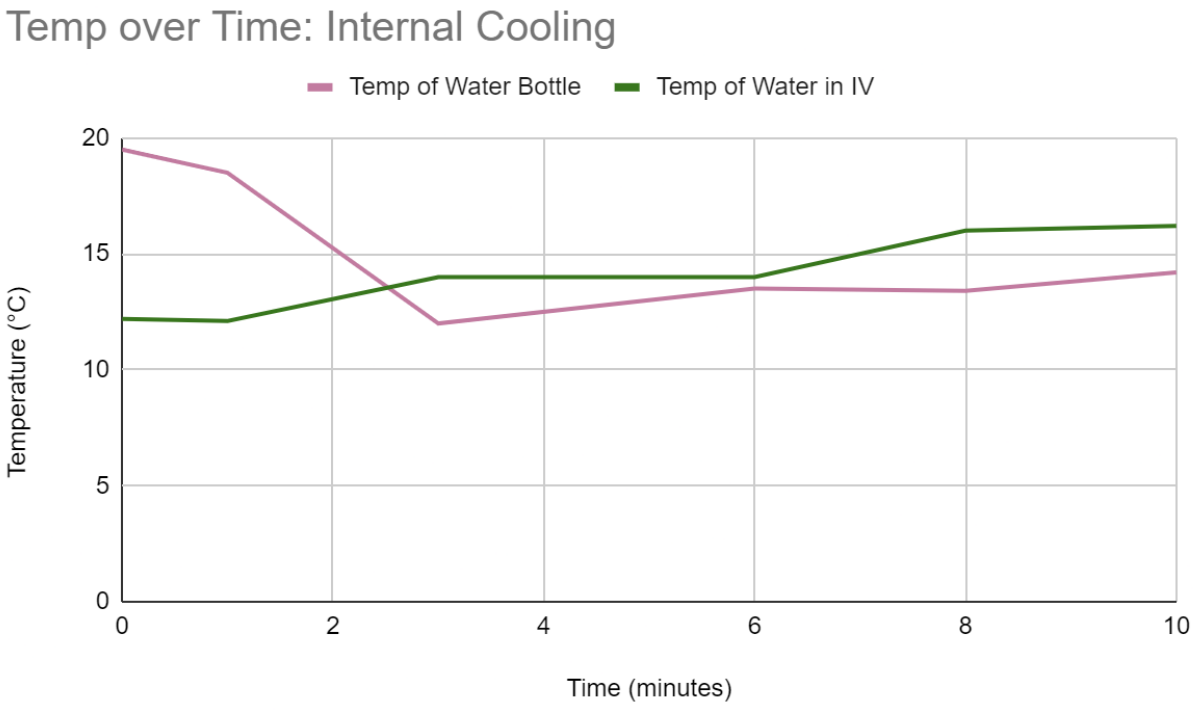


Figure 12 Table depicting time versus temperature for the perfusate experiment.

The graphs in Figures 11 and 12 show that the perfusate experiment cooled significantly faster. However, an important limitation to highlight for this experiment was that the ambient temperature of the room where the experiment was performed was colder than an average room temperature setting. This can be seen in the starting temperatures of the parts of the experiment, so the cooling was only a few degrees versus the actual application of the device from a regular body temperature of about 37 °C.

The most desirable trait of this design was that it would be applicable for either amputation situation, whether it was fully amputated or partially amputated. It also demonstrated the ability to rapidly cool to the target temperature within minutes of being attached (on a smaller scale). There are no electrical components necessary for this device to work as it only requires gravity. The design is compact and simple and could keep the limb cooled for a long time. Lastly, the temperature is easy to maintain regulated to ensure it doesn't fall out of the necessary range. However, the user must be able to find a viable vein and artery to cannulate. This would require additional training for medical personnel that would be operating the device. Although this would be possible and strongly encouraged even for those who already know how to cannulate, it would require the use of extra resources. The other issue is that all the usual risks associated with cannulation still apply, including any dirt or bacteria that could occur if used at an amputation site. Lastly, if the transportation time requires a significantly longer amount of time, additional steps would be required to maintain the system. This would include attaching new perfusate bags when the previous one empties.

4.3.3 Ex Vivo Perfusion

Ex Vivo Perfusion is similar to the other cannulation methods. The materials needed for this procedure are two cannulas, an oxygenator, a centrifugal pump, and a tubing system. The procedure of an ex-vivo perfusate starts off with a tourniquet being applied above and below the amputation site. Then in an area of undamaged tissue below the amputation site the vein can be cannulated. This will allow for deoxygenated blood to go through a pump. The pump will provide the pressure needed for the blood to flow through the oxygenator, where deoxygenated blood will be exchanged into oxygenated blood. The oxygenated blood will flow through the second cannulation and into an artery. Thus, the oxygen supply is returned to the limb, preserving the limb. The justification for this process is a similar process called ECMO, where blood is pumped outside of the body to a machine that removes CO_2 and then sends back oxygenated blood to the tissues in the body.

Calculations for the determining the amount of surface area needed for proper oxygen exchange:

300 ml Blood/min
Cardiac output: 5,000-6,000 ml Blood/min
300/5,000 = 6%
300/6,000 = 5%
SA of Lungs: 70-140m
70(0.06) = 3.5 m^2
70(0.05) = 7 m^2
140(0.06) = 4.2 m^2
140(0.05) = 8.4 m^2
8 m^2 of SA = 8 x 10^6 mm^2

Some pros of this method are that the limbs will be highly viable during the transport time, the method has already been proven to work, it is effective immediately after attachment, and requires no maintenance after attachment. However, the cons of ex-vivo perfusion are that it is a large and complex device, requires many electrical components, is expensive, contamination is highly likely, and the process of cannulation can be difficult.

4.4 Final Design Selection

The decision to utilize CO_2 in the final device design instead of alternative methods (cannulation, perfusate, and ex-vivo perfusion) was made using a comprehensive analysis. This analysis compared various factors to identify the feasibility of each solution. These factors included the ability to increase the preservation time of the limb, the size and weight of the device, the ambient operating condition the device can work in, the time it would take for the device to cool the limb, the estimated final market price, the additional training required by medical personnel in order to safely and effectively use the device, and the ability for the device to work on any type of traumatic amputation (partial, total, crush, tear, etc.). These characteristics were assigned a weight based on the Pairwise Comparison Chart in Table 2. All the methods were then compared using Pugh Analysis. The final scale using this weight system was from –17 to 17. The results of this analysis were that the dry ice, perfusate, cannulation, and finally ex-vivo, with the scores being 10.25, 6.75, 5, and –4.5 respectively. Table 6 shows the results of this analysis. The ranking of this analysis was also validated by Doctor Giorgio Giatsidis MD, PhD, an assistant professor at UMass Chan Medical School and a plastic surgeon at T.H. Chan School of Medicine.

Table 6 Pugh Analysis

Requirement	Weight	Baseline	Dry Ice	Perfusate	Cannulation	Ex-Vivo
Preservation time	**3.5**	**0**	**1**	**1**	**1**	**1**
Size/Weight	**1.5**	**0**	**0.5**	**0.5**	**1**	**-1**
Ambient OP Condition	**2.5**	**0**	**1**	**1**	**1**	**0**
Time to Cool	**3**	**0**	**0**	**1**	**1**	**0**
Cost	**0.5**	**0**	**-1**	**0**	**1**	**-1**
Additional Training	**2**	**0**	**0**	**-1**	**-1**	**-1**
Universal Usage	**4**	**0**	**1**	**-1**	**-1**	**-1**
Total	**17**	**0**	**10.25**	**6.75**	**5**	**-4.5**

The reason that the CO_2 cooling sleeve comes in first is primarily its universal usage and lack of extensive training to EMS providers. The other designs are not able to be used for all types of amputation, specifically in crush and avulsion amputation. In these types of amputations there is a significant amount

of tissue damage. This would make it extremely difficult and dangerous to cannulate the blood vessels. Additionally in these types of amputations it would be impossible to see if there was extreme vascular damage resulting in noncontinuous blood vessels. Furthermore, the CO_2 cooling sleeve would not require any additional training whereas the other devices would require EMS providers to be trained on how to cannulate additional veins and arteries.

The initial design idea using CO_2 as a method of cooling involved having the limb being put inside of a bag or sleeve-type device and blasting CO_2 into that device. The first step in the process of prototyping this device was to identify what materials would make up the bag that would serve as an insulator of the limb from the outside environment. Different materials were identified based on their insulative properties, price, and volumetric characteristics, including factors such as bulkiness and mobility. This included neoprene and vacuum-metalized polyethylene. A weatherproof exterior layer was also required so this device could be used in all environments. The material chosen for this layer was a vinyl mesh. To test the insulative properties of these materials, thermistors were programed and calibrated. The thermistors were calibrated using boiling water and ice water and comparing the temperature outputs to a reference temperature probe that was already calibrated. The specific calibration procedure can be found in Appendix B, the code used to program the Arduino can be found in Appendix C, the circuit diagram can be seen in Figure 13, and the calibration data can be found in Table 7.

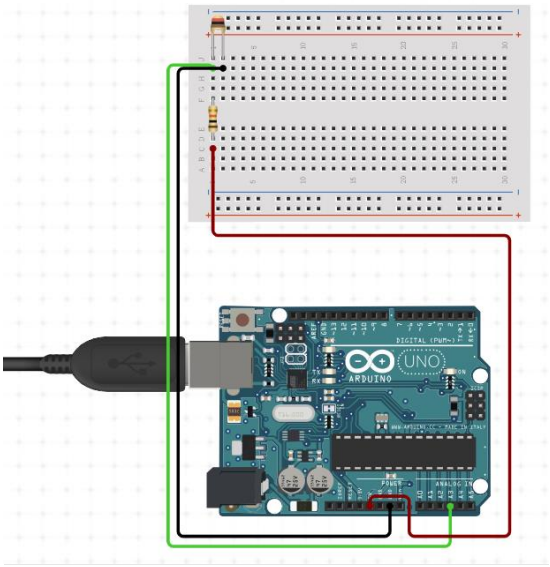


Figure 13 Arduino Uno set up for temperature readings.

Table 7 Temperature Probe Calibration Testing

Temperature Probe 1 (DATE)							
Probe	Thermometer Used	Hot Bath		Cold Bath		Calibration	
		C	F	C	F	C	F
A	Reference Temp	99.99	212	0.5	33.3	y=0.882x+3.12	y=0.886x+9.69
	Test Temp	109.77	228.43	-2.97	26.66		
B	Reference Temp	100	211.8	6.5	43.2	y=0.87x+6.16	y=0.871x+14.7

	Test Temp	107.88	226.19	0.39	32.7		
C	Reference Temp	99.2	210.4	0.4	32.9	y=0.862x+0.452	y=0.86x+5.56
	Test Temp	114.57	238.23	-0.06	31.8		

Once the thermistors were calibrated, the materials were tested. The neoprene material used was a neoprene sponge foam rubber sheet, specifically a closed-cell foam. It was sourced from Amazon and the width, length and thickness were 12 in, 59 in, and 0.39 in, respectively. This roll was then cut into two sections with the dimensions to 48.25 cm ± 0.05 cm by 30.5 cm ± 0.05cm and 48.25 cm ± 0.05 cm by 11.1 cm ± 0.05cm. A circular piece with a diameter of 26. 5cm ± 0.05cm was also cut. The vacuum-metalized polyethylene was an emergency blanket sourced from Walmart. The blanket was folded so that there were two layers approximately 0.54 mm ± 0.01 mm thick. This material was then cut to dimensions 48.25 cm ± 0.05 cm by 49 cm ± 0.05 cm, as well as another circular piece with diameter of 26.5 cm ± 0.05 cm. The vinyl mesh bag was a 20 L dry bag sourced from Walmart™. The dimensions of the bag are 25.3 cm ± 0.05 cm wide by 54.4 cm ± 0.05 cm long by 1.59 mm ± 0.01 mm thick.

The insulative properties of the material were tested by putting a bag of ice water inside the prototype and waiting for 48 minutes. The time was selected because it took 48 minutes for the control, ice water in a bag sitting on the table, to reach room temperature. Approximately 150 ml of water and 150 ml of ice were put into the sandwich sized Ziploc bag. There were four different material combinations tested: just the dry bag, the dry bag and the cut neoprene fit inside of it, the dry bag and the cut emergency blanket (e-blanket) fit inside of it, and finally the dry bag with both the e-blanket, and neoprene inside (dry bag & all the materials). The temperature change slope was used to identify the design's effectiveness, with a higher slope meaning less effective insulation and a lower slope meaning more effective insulative properties. The results of this testing were that the dry bag with both the neoprene and e-blanket was best followed by the dry bag with the e-blanket, the dry bag with the neoprene, and finally just the dry bag. The slopes were 0.0742, 0.789, 0.121, and 0.205 respectively. These results can be seen in Figure 15 and the diagram of the material layering can be seen in Figure 14.

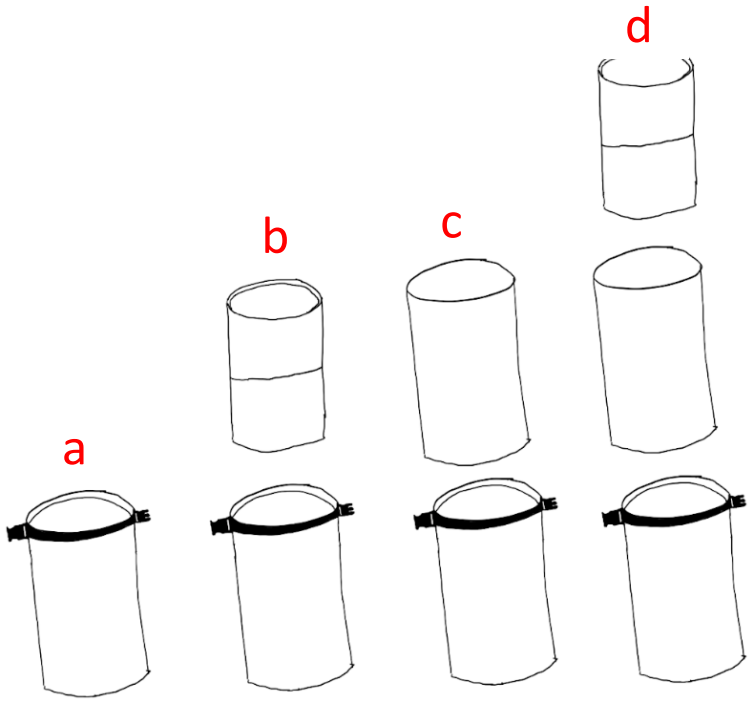


Figure 14 Diagram of material layering for initial material testing (a) Dry bag (b) Dry bag and neoprene (c) Dry bag and e-blanket (d) Dry bag and all the materials.

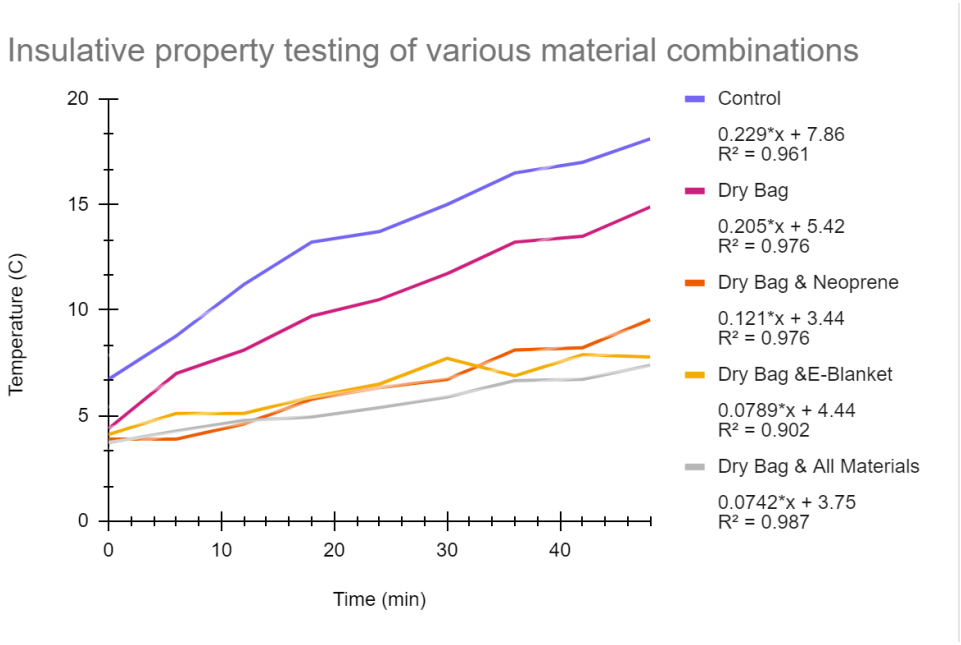


Figure 15 Experimental testing of outer material's ability to insulate device.

After the materials used in the outer, insulative layer of the device were selected, the material of the layer in contact with the limb needed to be selected. Studies done in the previous year's project have shown that the direct contact of the limb to dry ice, especially between the limb and the cooled air, resulted in frostbite. To prevent frostbite from developing, a contact layer was researched. The initial design idea utilized a gel bead sleeve with an internal bag system. It was hypothesized that the gel bead contact layer would exhibit a prolonged cooling period compared to air, attributed to its higher heat capacity. Consequently, this would maintain the skin temperature at a consistently warmer level than the surrounding cooled air, while still adequately cooling the limb. The internal bag system was designed to keep the dry ice snow close enough to the contact layer that heat transfer would not be dramatically slowed. There were four different designs that were tested during the experiment. The first design contained the gel bead contact layer, the internal bag system, and the insulated bag (including neoprene, e-blanket, and the dry bag layer with tubing). The next design was the gel bead contact layer and the insulated bag. The final two designs were the internal bag system and the insulated bag as well as the insulated bag with no contact layer or internal bag system. The diagrams of the device's design can be seen in Figure 16.

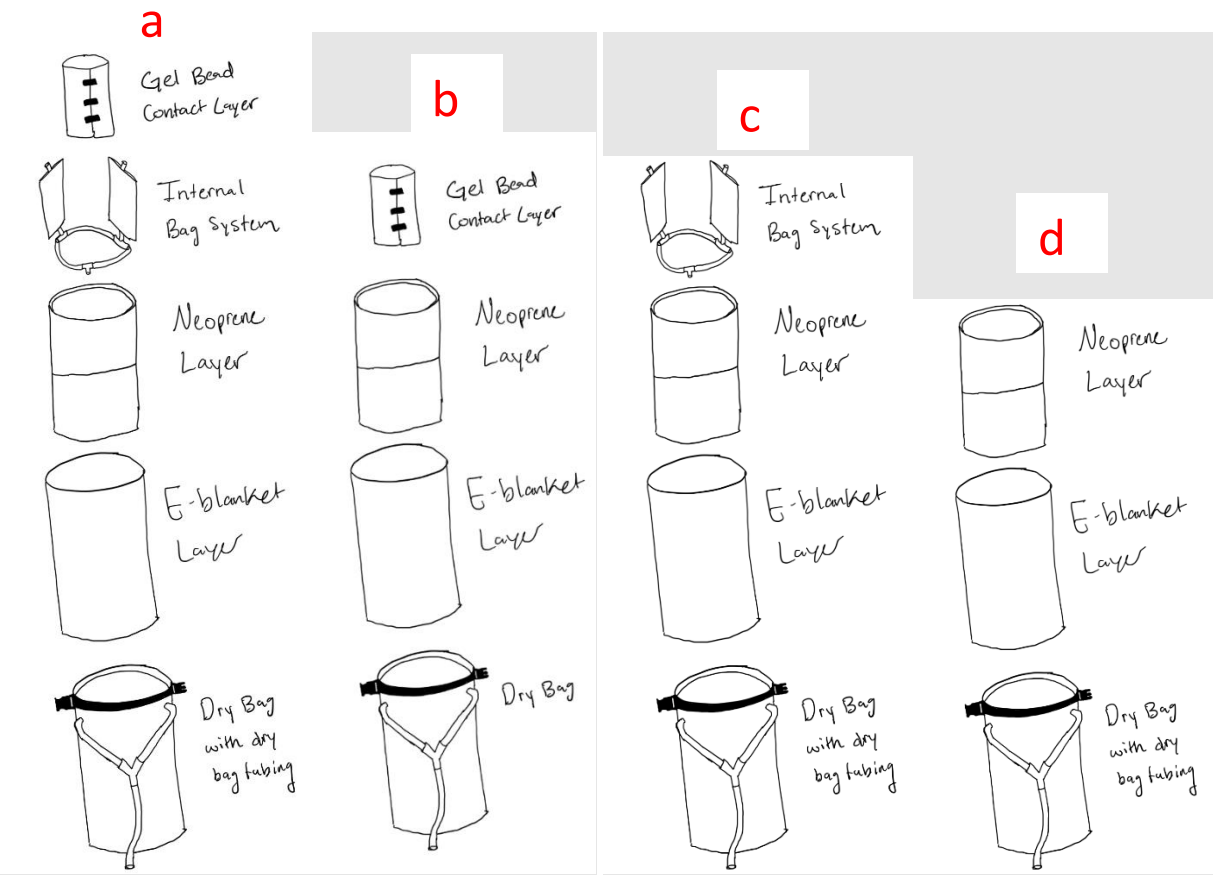


Figure 16 Exploded view of device design concepts. (a) Design 1: Contact Layer and Internal Bag System (b) Design 2: Contact Layer and No Internal Bag System (c) Design 3: No Contact Layer and Internal Bag System (d)Design 4: No Contact Layer and No Internal Bag System

Two different gel beads were tested for the contact layer: Orbeez ™ and the beads found in the *Hot + Cold Gel Bead Compress* by up & up™. Two different batches of Orbeez were used: one soaked for 15 minutes, and one soaked for 50 minutes. The gel beads samples were vacuum sealed using the moist setting in plastic bags. The up & up ™ gel beads, Orbeez ™ 15 min, Orbeez ™ 50 min vacuum sealed bags with dimensions of 26.2 ± 0.05 cm cm by 26.1 cm ± 0.05 cm, 8.4 cm by 26.1cm ± 0.05 cm, and 6.6 cm ± 0.05 cm by 26.1 cm ± 0.05 cm respectively. The thickness of each were also measured to be 4.4 mm, 4.6 mm, and 5.5 mm respectively. Images of each bag can be seen in Figure 17.

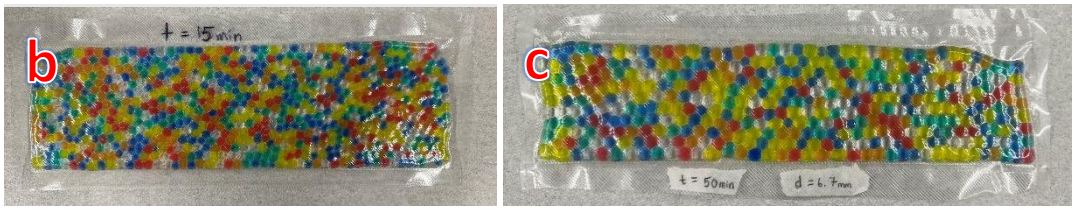


Figure 17 (a) up & up ™ gel beads in vacuum sealed bag (b) Orbeez ™ soaked for 15 min in vacuum sealed bag (c) Orbeez ™ soaked for 50 min in vacuum sealed bag.

Once the gel bead packs were made, they were placed overnight (~24 hours) in a freezer kept at approximately -18 ºC (0 ºF) to test the properties of the gel beads at subzero temperatures. The results of this were that the up & up ™ gel beads were very cold to the touch, they did not stiffen, and they were still very flexible. Both Orbeez ™ packs demonstrated freezing between gel beads and freezing in entire gel bead clusters. The gel packs were then cooled down to room temperature and the up & up ™ gel beads remained the same. The Orbeez ™, however, seemed to shrink or burst. The Orbeez ™ 15 min pack seemed to mostly shrink with some minor bursting. The Orbeez ™ 50 min pack demonstrated mostly bursting and most of the pack was turned into a mushy jelly-like consistency. The pack itself was very solid and not flexible at all. Therefore, it was found that the up & up ™ gel beads were superior to the Orbeez ™ as they need to withstand the temperatures of -78 degrees C (-109 F) and remain flexible when they contact the dry ice. Images of the gel bead packs directly after freezing and after cooling back to room temperature can be seen in Figure 18.

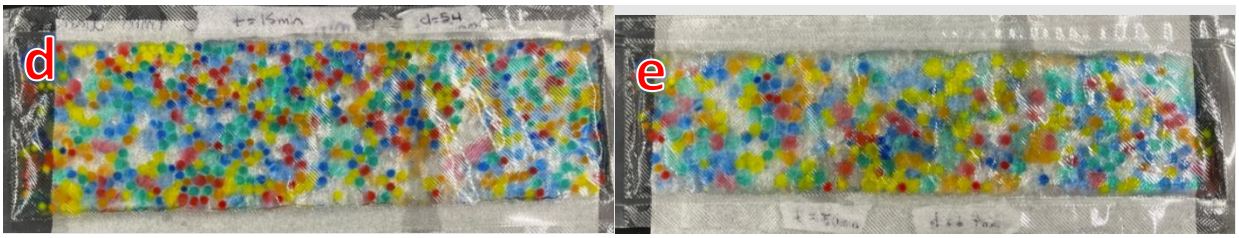


Figure 18 (a) up & up ™ gel beads in vacuum sealed bag after freezing for 24 hours (b) Orbeez ™ soaked for 15 min in vacuum sealed bag after freezing for 24 hours (c) Orbeez ™ soaked for 50 min in vacuum sealed bag after freezing for 24 hours (d) Orbeez ™ soaked for 15 min in vacuum sealed bag after defrosting (e) Orbeez ™ soaked for 50 min in vacuum sealed bag after defrosting.

After the up & up ™ gel beads were selected, the contact layer was made using two, gallon sized plastic bags vacuum sealed with the gel beads inside. The next step of designing the prototype was the creation of the internal bag system. The initial design of the internal bag system involved taking a gallon sized plastic vacuum sealable bag and cutting a hole at the bottom about 1.5 inches big. The perpendicular hole of a 17.67 mm ± 0.01 mm internal diameter T connector was then secured in place using the gorilla glue adhesive. The top of the bag was then sealed with just the seal setting of the vacuum sealer leaving a 1.5 in gap in the center. Here, a 2 in long 22 mm ± 0.01 mm diameter sized tube was inserted into the hole and secured with gorilla glue adhesive. Two of these bags were made. Three additional pieces of this tubing were cut to be 11 in. These pieces were attached with one end on either parallel connection of the T connector secured to the bag and one T connector not secured to the bag, with the perpendicular connection facing in the opposite direction. This can be seen in Figure 19. The tubing attached to the top of the bags was then attached to a 12.22 mm ± 0.01 mm socket connection and a 1 in long piece of 15 mm internal diameter tubing was attached in parallel to a 23.54 mm ± 0.01 mm internal diameter y connector. Another length of that tubing was cut to be 2 in long. This was then put on the final connection of the y connector. A three foot long, 22 mm ± 0.01 mm internal diameter tubing was then attached to that so that it could be fitted directly to the CO2 tank. The connections were temporarily secured with duct tape to prevent CO2 from escaping. This can be seen in Figure 20. The fully built internal bag system can be seen in Figure 19.

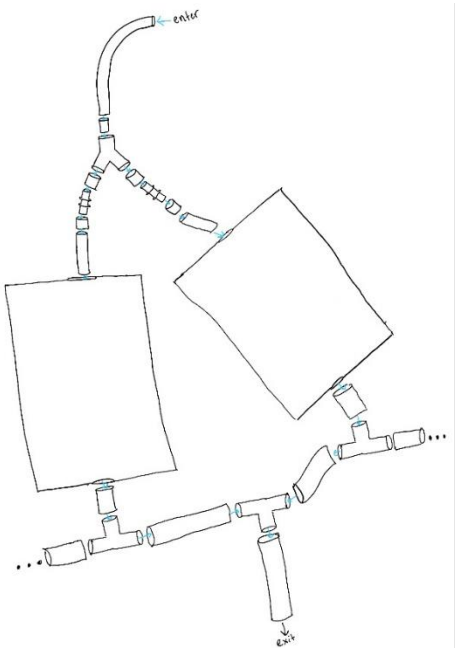


Figure 19 Diagram of the internal bag system.

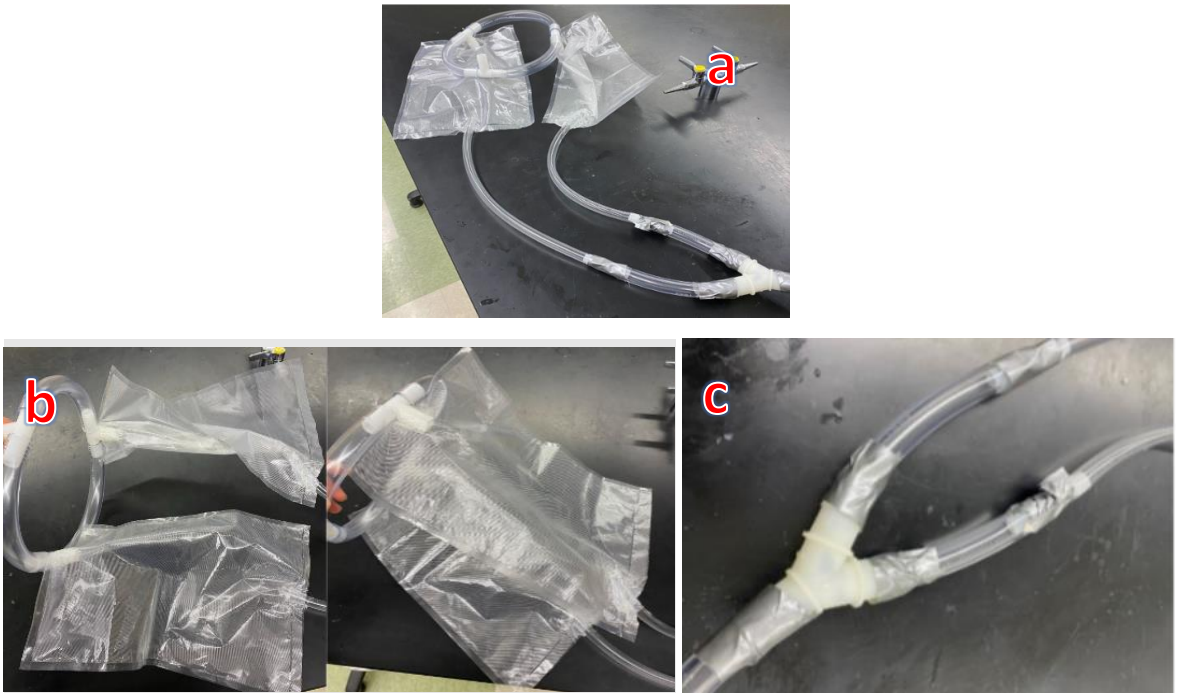


Figure 20 (a) Entire bag system set up (b) Lower assembly of bag system viewed at two different angles (c) Upper assembly of bag system y-connection.

Testing the internal bag system involved the use of a systematic procedure. First, all persons put on personal protective equipment including insulated gloves, safety glasses, and a lab coat. Next, the

tubing seen at the top of the internal tubing diagram in Figure 19 was connected securely to the CO2 tank that had a siphon tube. With caution, the CO2 tank was opened to allow the device's internal mechanisms to fill with CO_2 gas as well as dry ice snow. Using a gloved hand, pressure was applied gradually to the bags, simulating operational conditions, while vigilantly monitoring the whole system for any leaks. Leaks can occur in the form of air or dry ice particles escaping the system. Air should only be escaping out the exit tube and there should be no dry ice snow coming out of the exit tubing and minimal dry ice in the tubing downstream from the bags. During testing, it is imperative that the tubing remains free of kinks. If kinks occur, it can lead to blockages caused by the freezing of the tubes. The CO_2 tank was then closed and the distribution of dry ice snow into both bags was verified. By adhering meticulously to this step-by-step procedure outlined in Appendix B, the integrity and functionality of the internal bag system can be assessed, guaranteeing optimal performance and safety when used inside the device. The result of this testing was that the bags would indeed be able to withstand the environment inside of the device and work properly. It was also observed that there was an equal amount of dry ice snow in each bag. In Figure 21a the experimental set up can be seen as well as images of the bags inflated (Figure 21b) and deflated after the testing (Figure 21c).

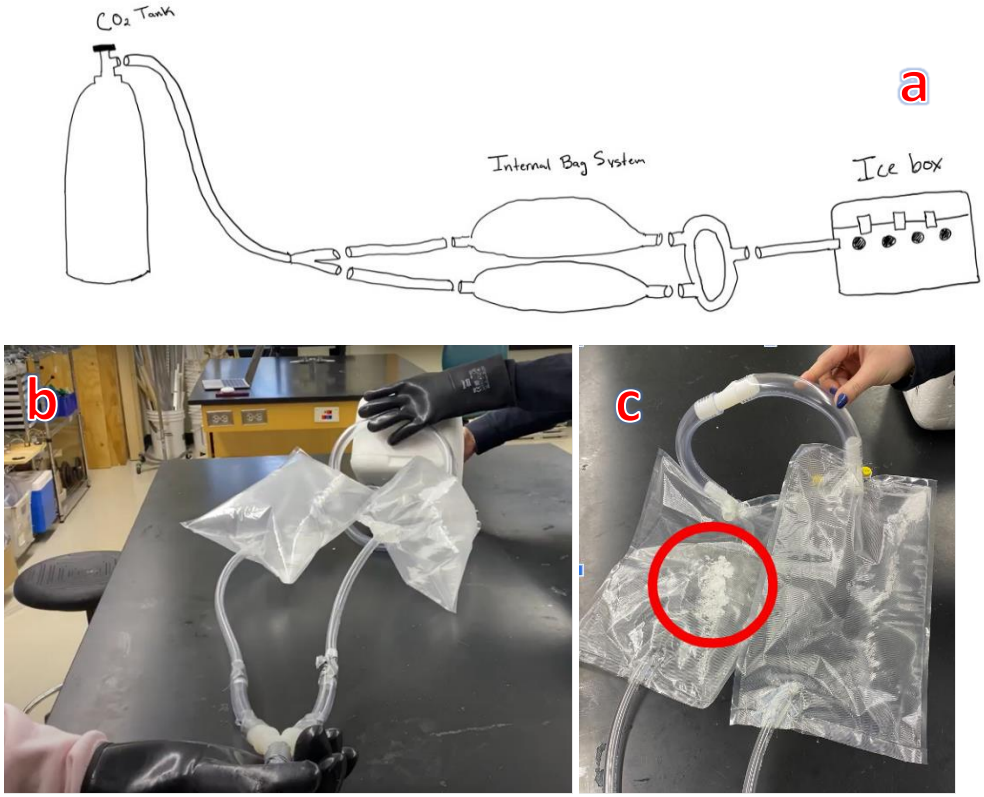


Figure 21 (a) Experimental set up of internal bag system testing (b) Image taken during testing with internal bag system fully inflated (c) Image taken after internal bag system testing.

The result of this experiment was that the bags were able to withstand the pressures exhibited by the CO_2 tank. Minor changes to the internal bag system were made in the preliminary testing such as increasing the diameter of the exit tube. Furthermore, the duct tape used to hold the tubes together in the preliminary experiment was changed to hot glue. The gorilla glue was also replaced with hot glue as it did not properly adhere the bag together. Now that all the parts of the device were assembled and preliminary tests were completed, the device designs had to be tested. To test the device designs the amount of dry ice

released from the tank had to be calculated. Safety measures were ensured, including the use of proper personal protective equipment (PPE), and adequate ventilation. Liquid CO_2 was then gradually released into the bin for various time intervals and the weight of the bin was recorded before and after the CO_2 was dispensed. This process was repeated three times for each trial, and it was determined that the time dispensed does not change the rate of CO_2 dispensed. Thus, the average rate was found to be 5.4 grams/second. The data collected during this experiment can be found in Table 8, more information on the experimental procedure can be found in Appendix B, and the experimental set up can be seen in Figure 22.

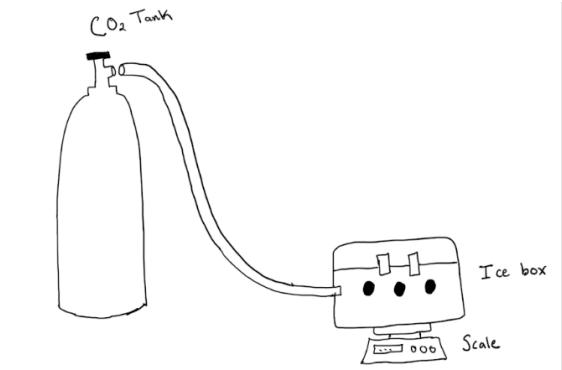


Figure 22 Experimental set up CO_2 tank rate of release testing

Table 8 CO_2 tank rate of release experimental data

Time (s)	Trial	Actual time (s)	Weight of Dry Ice (g)	Rate (g/s)	Rate (g/s)	Avg. Rate (g/s)
10	1	10.64	53.2	5.0	5.6	5.4
	2	10.49	65.4	6.2		
	3	10.65	59.5	5.6		
15	1	15.17	64.2	4.2	4.0	
	2	15.31	57.5	3.8		
	3	15.88	61.6	3.9		
20	1	20.58	140.2	6.8	6.2	
	2	20.4	137.1	6.7		
	3	20.83	106.2	5.1		
30	1	30.85	120.1	3.9	5.9	
	2	30.52	183.9	6.0		
	3	29.55	230.3	7.8		

The final device designs underwent testing using a method involving Jello contained within a 2-liter bottle, chosen for its similarity in heat capacity to that of a human limb, ensuring a realistic cooling simulation. The testing procedure started by placing thermistors at the center of the Jello and taping one to the outside surface of the 2-liter bottle. The Jello was then warmed using a laboratory oven to an average human body temperature of 37°C before being inserted into the device design being tested. Four designs were tested: no contact layer and no internal bag system, no contact layer and internal bag system, contact layer and no internal bag system, contact layer and internal bag system. The entrance was attached to a CO2 tank, and the tank was opened for 10 seconds every five minutes. This time interval was determined by research conducted by the previous year's team. This process continued until the internal temperature of the device reached 16°C, the designated target temperature. This signaled the end of the experiment. Throughout the testing, safety measures were strictly adhered to. The device was also sealed shut during the experiment with tape to simulate the actual usage case. The experimental set up can be seen in Figure 23 and more information on the procedure can be found in Appendix B.

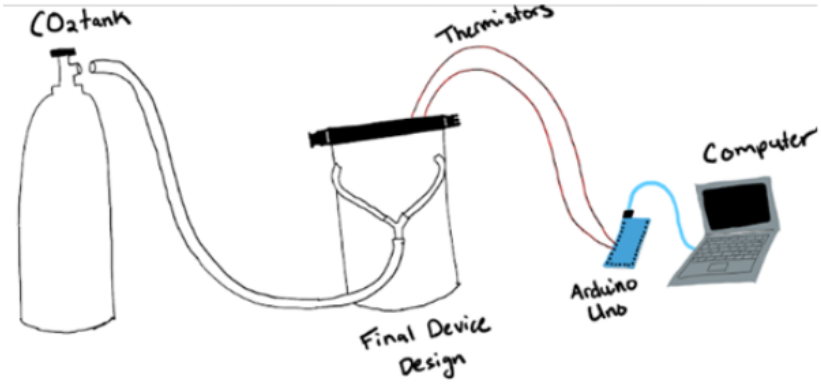


Figure 23 Experimental set up of device design testing

The first designs that were tested were those without the internal bag system. The no contact layer no bag system design decreased in temperature at a rate of 0.298 ºC per minute. During this experiment the surface temperature was in subzero temperatures for a total of 26 minutes of the 35-minute experiment. Thus, for approximately 74% of the experiment the temperature was in subzero temperatures with the longest consecutive time below 0 ºC being 24 minutes. The second design tested utilized no bag system with a gel contact layer. This design caused the temperature to decrease at a rate of 0.25 ºC per minute. A total of 17 minutes out of the 40-minute experiment the surface temperature of the Jello was measured to be subzero. Approximately 38% of the experiment the surface temperature of the Jello was below 0 ºC and the longest consecutive time in subzero temperatures was 4 minutes. The Jello during this experiment was found to be frozen after the testing was completed. Thus, it was determined that the design involving no contact layer was more effective, even with the longer subzero surface temperature, as this can most likely be adjusted in further testing and refining of the experiment. The data regarding these two designs can be found in Figure 24.

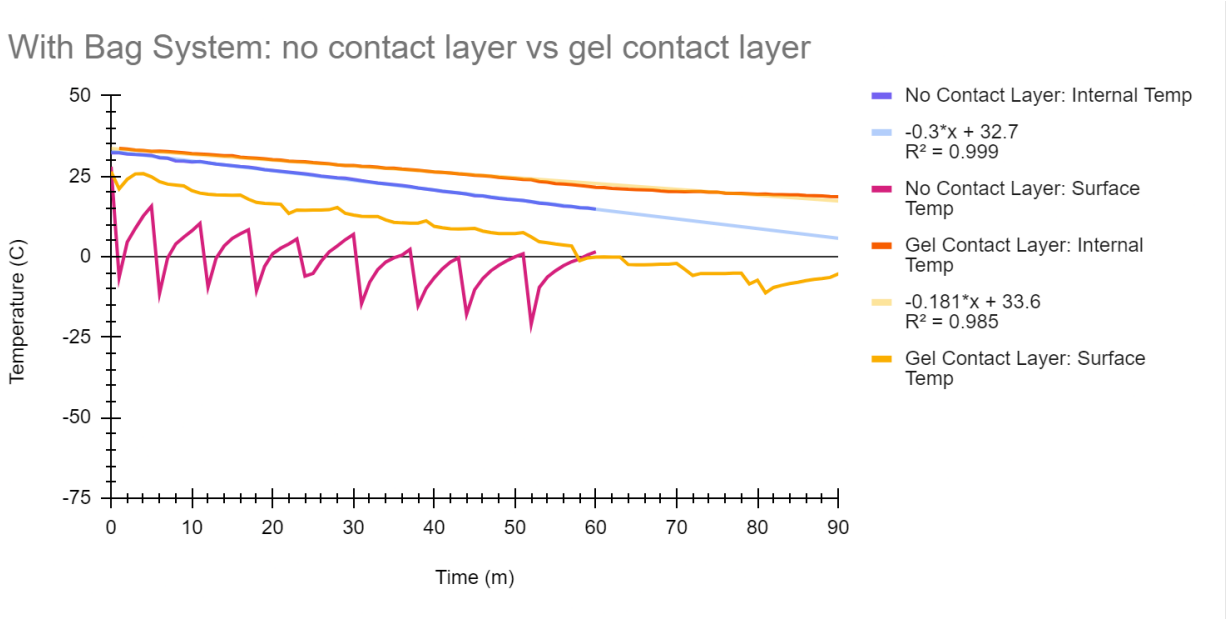


Figure 24 Experimental evaluation of device designed with and without a gel bead contact layer.

The second designs that were tested were those with the internal bag system. The no contact layer no bag system design decreased in temperature at a rate of 0.3 ºC per minute. The surface temperature was subzero temperatures for a total of 35 minutes of the 60-minute experiment. Thus, for approximately 58% of the experiment the temperature was in subzero temperatures. The longest consecutive time below 0 ºC was 13 minutes. The second design utilized the internal bag system and a gel contact layer. This design caused the temperature to decrease at a rate of 0.181 ºC per minute. A total of 36 minutes out of the 92-minute experiment the surface temperature of the Jello was measured to be subzero. Approximately 38% of the experiment the surface temperature of the Jello was below 0 ºC and the longest consecutive time in subzero temperatures was 36 minutes. The Jello during this experiment was also found to be frozen after testing. Therefore, the team determined that the design involving no contact layer was more effective. The data regarding these two designs can be found in Figure 25. The rate of cooling from each design was compared. The final best two designs were the no contact layer designs with and without the internal bag system. The cooling rate of these designs were 0.3 ºC per minute and 0.298 ºC per minute respectively. The best design was determined to be the no contact layer with the internal bag system.

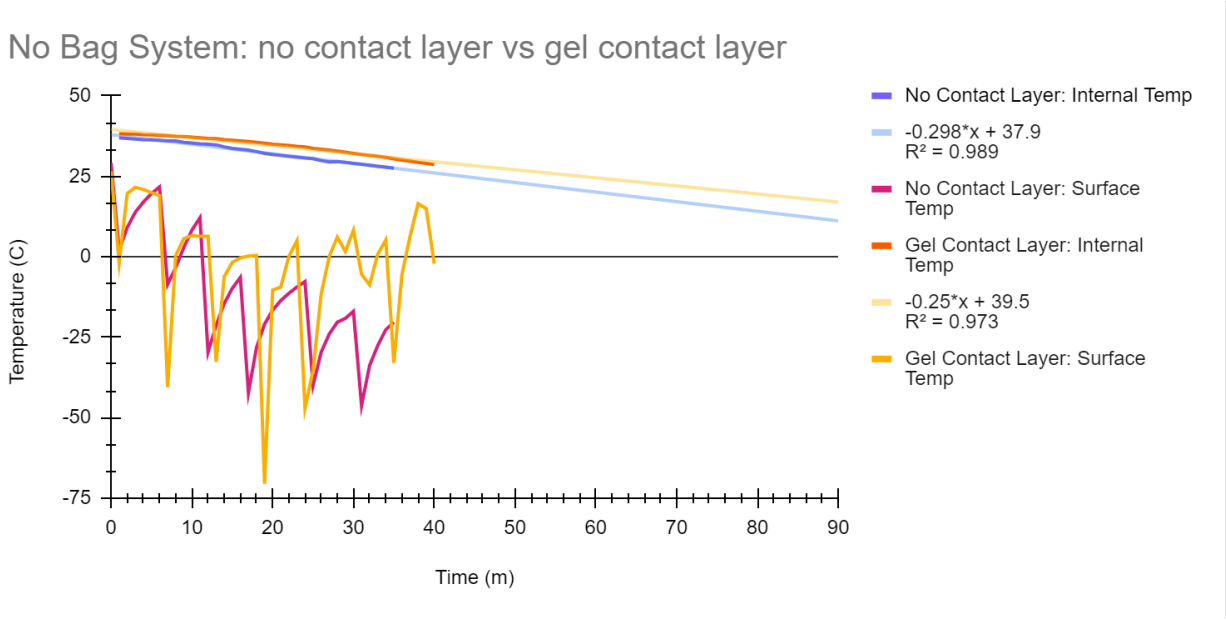


Figure 25: Experimental evaluation of device designed with bags to contain CO2 with and without gel bead contact layer.

After all the device designs were tested, the state-of-the-art method for cooling traumatically amputated limbs was tested. The state-of-art method, in short, is wrapping the limb in a saline solution-soaked bandage and putting in it a bag surrounded by ice water. This method was tested by first placing thermistors in the center of the 2-liter bottle filled with Jello. The Jello was then warmed to an average human body temperature of 37°C. A large bucket was filled with one part water and one part ice (approximately 2-liters of each). The 2-liter Jello was then placed in a plastic bag and then into the bucket of ice water. It was made certain that the 2-liter was completely submerged in the ice water. The internal temperature of the 2-liter filled with Jello was recorded until it reached the goal temperature. It was found that the temperature decreased by 0.195°C per minute. The experimental set up of the state-of-the-art testing can be found in Figure 26 and the results of the experiment can be found in Figure 27.

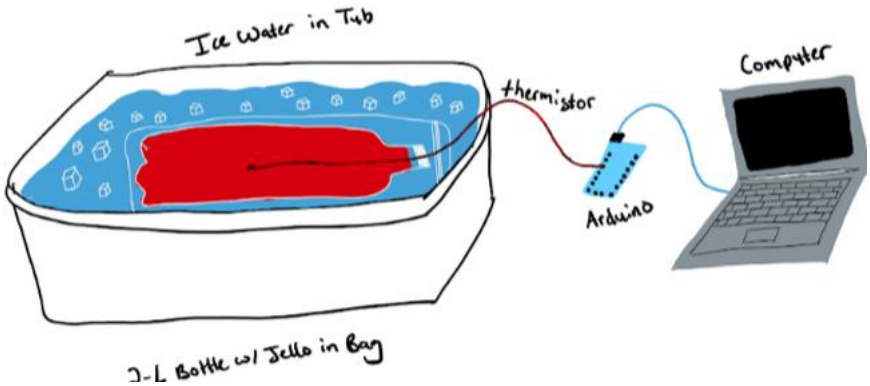


Figure 26 Experimental set up of state-of-the-art testing

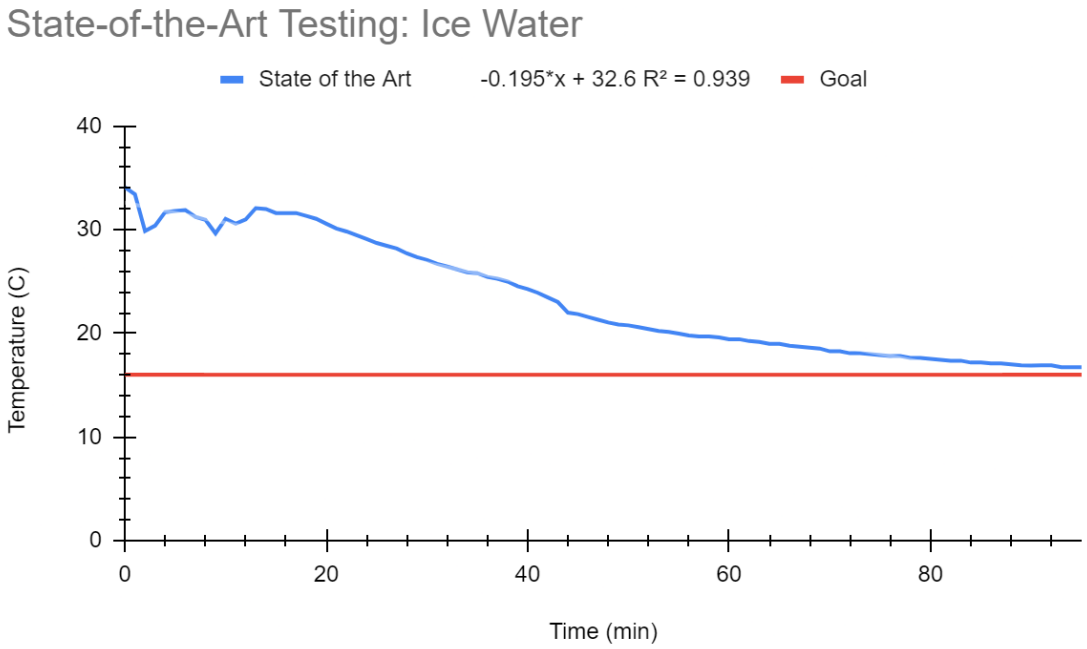


Figure 27 Experimental evaluation of State-of-the-art: Ice water in bag

To be certain that the final device design was comparable to the state-of-the-art method, the no contact layer with bag system was compared to the state-of-the-art method. It was found that the rate of cooling of the final device design—no contact layer with bag system— was over 1.5 times more effective than the state-of-the-art. The slopes of them being 0.3°C per minute compared to 0.194°C per minute, respectively. The comparison between these two methods can be seen in Figure 28.

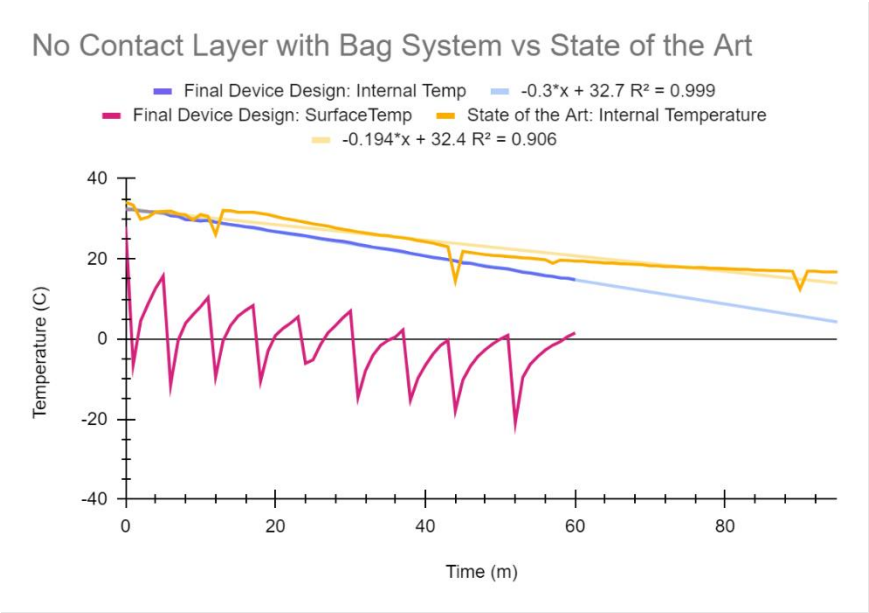


Figure 28 Experimental evaluation of CO2 device in comparison to the state-of-the-art device.

Comparisons with the state-of-the-art device, water and ice in a bag, further validate the superiority of the CO_2 -based approach. Cannulation, while theoretically addressing ischemia, is deemed impractical and unsafe due to the complexity of vascular reconnection and the associated risks. Similarly, perfusate presents challenges related to risk factors and contamination. Ex-vivo perfusion is dismissed due to its impracticality, large size, and high cost. In addition to the comparisons highlighted in the Pugh analysis chart seen in Table 6, the experimental evaluation done has further reinforced the decision to opt for the CO_2 -based cooling method over alternative approaches. The state-of-the-art device employing water and ice in a bag serves as a benchmark, and the Pugh analysis affirms the dry ice approach's superior performance across multiple criteria. In summary, the holistic consideration of data and studies underscores the advantages of the chosen CO_2 -based cooling method, offering a practical and effective solution for emergency limb preservation.

The final device design of no contact layer and internal bag system will be employed by inserting the limb into the cavity of the bag. The external tubing will then be connected to a CO2 tank that contains a siphon tube. Dry ice snow would then be blasted into the internal bag system which would ultimately cool the limb. The pros of this design are that it quickly and cools the model limb more effectively than the state-of-the-art. The device is also very compact and able to be rolled up when not in use. Furthermore, this device has a much longer shelf life as the CO2 tank, unlike ice and water, can be stored for many years. The cons are that the CO2 tank is heavy and bulky. It also causes the surface temperature to be subzero, however, this can most likely be fixed with procedural testing. CO2 tanks are also much more expensive than ice.

Chapter 5: Design Verification

After finalizing a design for the device, the design specifications needed to be tested. A series of testing procedures were created to do this and are presented below. Through research, it was found that Jello has similar thermal conductivity characteristics to human skin. Jello was then used for preliminary testing, and a pig leg was used for further validation to simulate a human limb more closely.

5.1 Preliminary Verification Testing

Preliminary testing for the device was done using a two-liter soda bottle filled with Jello. This is due to the similar thermal conductivity properties that Jello has to skin. The thermal conductivity of Jello was found to be between 0.28 to 0.30 $Wm^{-1}k^{-1}$ while that of skin is 0.32 to 0.50 $Wm^{-1}k^{-1}$ [51, 52]. The plastic material of the bottle, polyethylene terephthalate, serves as a substitution for fat in the body while the Jello acts as the muscles. To have a baseline, a state-of-the-art test was performed for six hours using the Jello bottle. The bottle of Jello was placed in ice water and the temperature was monitored until it hit the target of 16 degrees Celsius. This took just over 90 minutes to reach and can be seen in figure __.

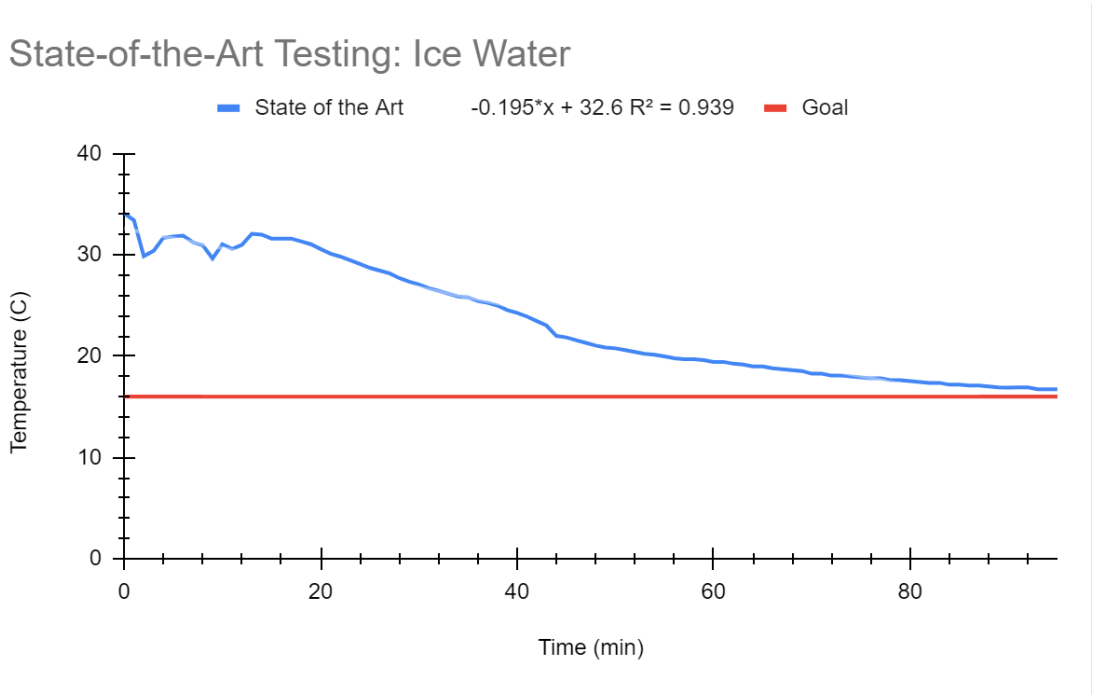


Figure 29. Temperature versus time graph of state-of-the-art: Jello bottle placed in ice water.

To test the validity of the device, several experiments were set up to test the device's functionality for extended periods of time (six hours) to meet the design specification. The initial setup for the longevity test involved the bottle of Jello, three thermistors connected to an Arduino, a small space heater, and the device placed under a hood. The Jello was warmed to about 37°C using an incubator. A CO_2 tank was placed right outside for the tubing connection that exits through the back of the hood. One thermistor was placed in the center of the bottle, another on the outer surface of the bottle, and the third was placed in the ambient environment of the hood. The thermistor in the center was there to monitor the

inner most temperature to ensure the entire model reached the target temperature. The surface thermistor was used to monitor the frostbite on the surface of the skin. Lastly, the ambient thermistor was used to measure the temperature of the environment under the hood to simulate hot climates such as deserts. The CO_2 was blasted in intervals of ten seconds every five minutes until the internal temperature of the bottle reached the target temperature of 16°C. Temperature values were recorded every five minutes for a duration of six hours. Figure 30 shows the experiment's setup and figure 31 shows the thermistor placements on the surface and center of the Jello and pig leg models. A complete procedure can be found in appendix E.

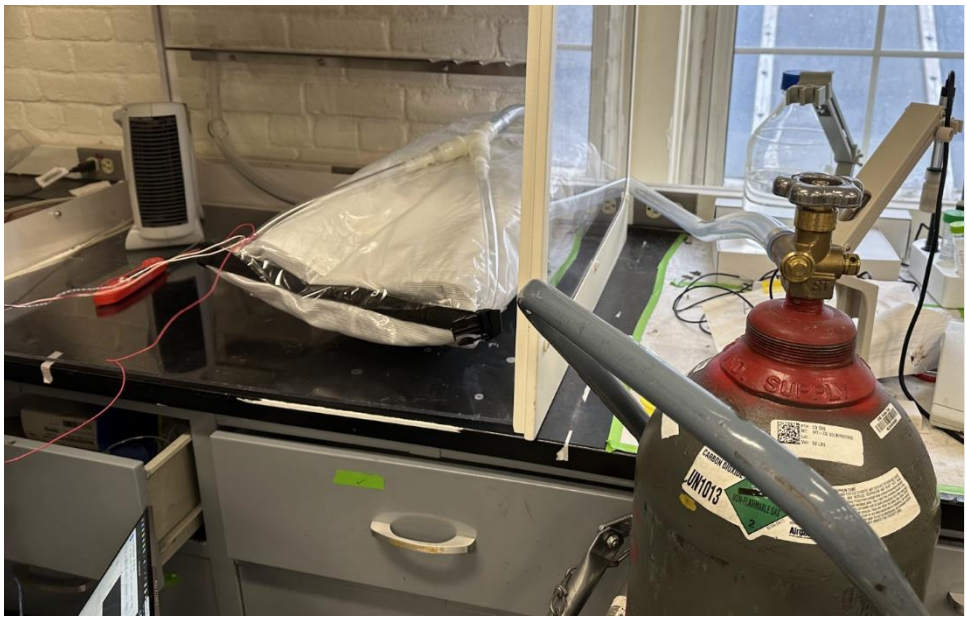


Figure 30. A setup of the experiment using the two-liter soda bottle for longevity testing of six hours.

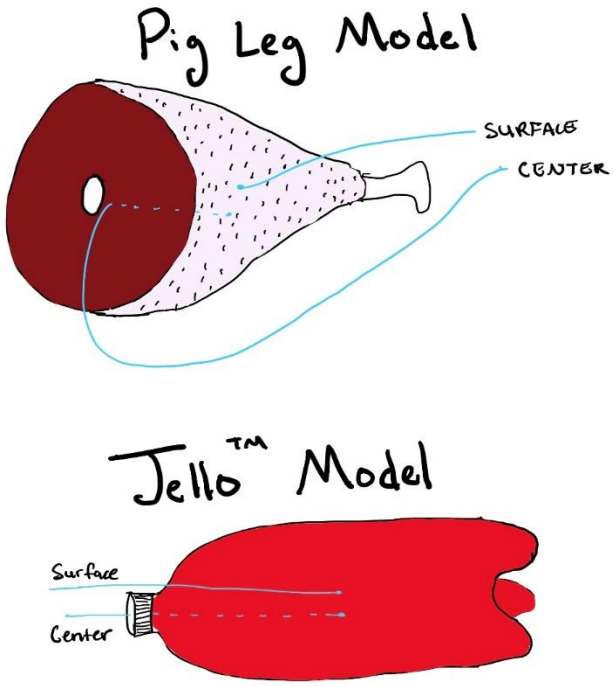


Figure 31. Diagrams of the thermistor placement for the Jello and pig leg models.

For this initial longevity experiment, the temperature reached 16.82°C in 65 minutes and only required CO_2 blasts for the first 60 minutes. The temperature remained on a steady decrease until 165 minutes (about 3 hours) when it reached its lowest temperature of 9.83°C and slowly started to rise again. At the conclusion of the six-hour test, the temperature was 15.24°C. This served as a baseline for the functionality of the device. For this initial experiment, there was no heater used for ambient temperatures. The data for the test can be seen in Figure 31.

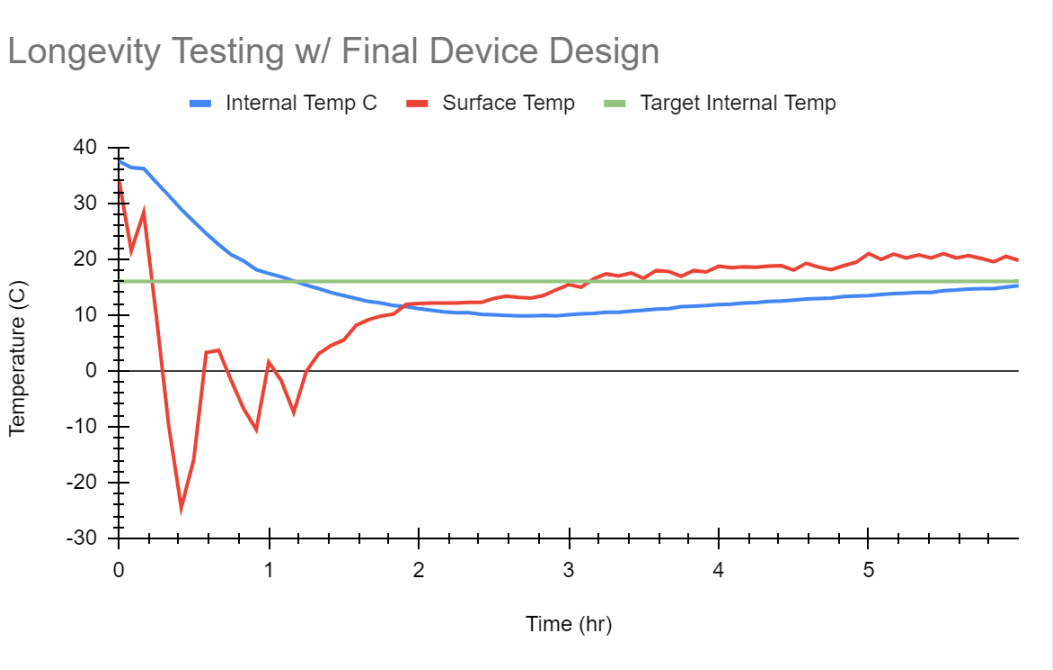


Figure 32. Graph showing the temperature data of the six-hour test with the Jello bottle.

The longevity test with the Jello bottle was then replicated a second time to strengthen the results received from the first test. For this experiment, an automatic heater was introduced and set to a constant heating temperature of 95 degrees Fahrenheit. The results in figure 33 represent the internal, surface and ambient temperatures of the Jello model.

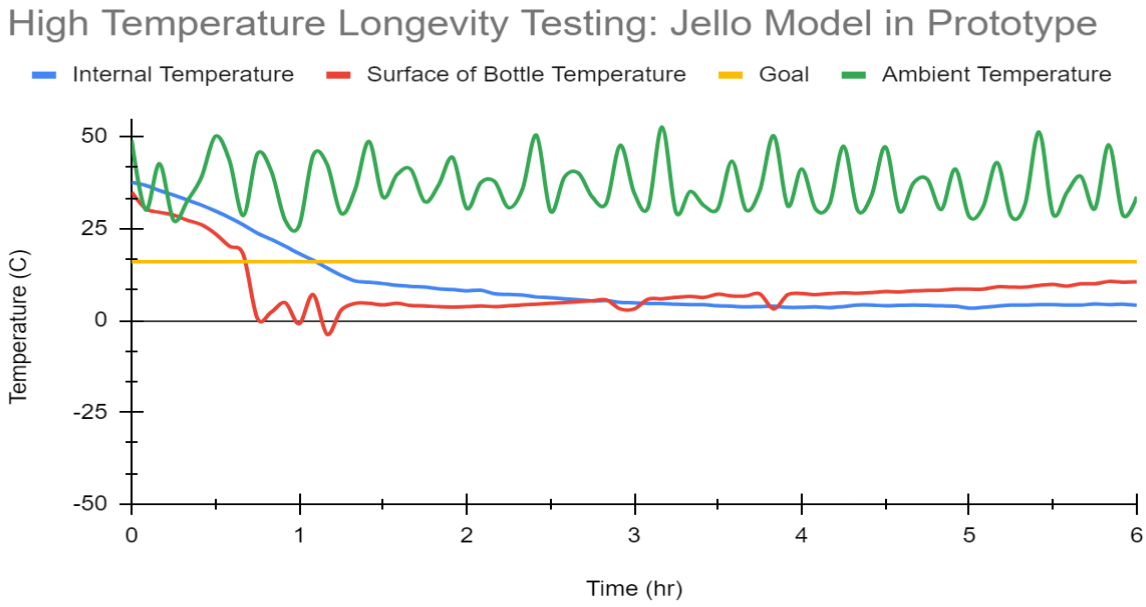


Figure 33. Graph showing temperature versus time data for second six-hour testing using a Jello model.

The final test performed for design verification was using a 14-pound pig leg with hip with the skin left on. The skin was kept intact to simulate a human leg as closely as possible, and a pig leg was used for the similarity to the anatomy and size of a human leg. However, the hair on the pig leg was shaved off to avoid interference. The setup for this experiment was done the same as the previous longevity experiment. Figure 34 shows the results of this experiment.

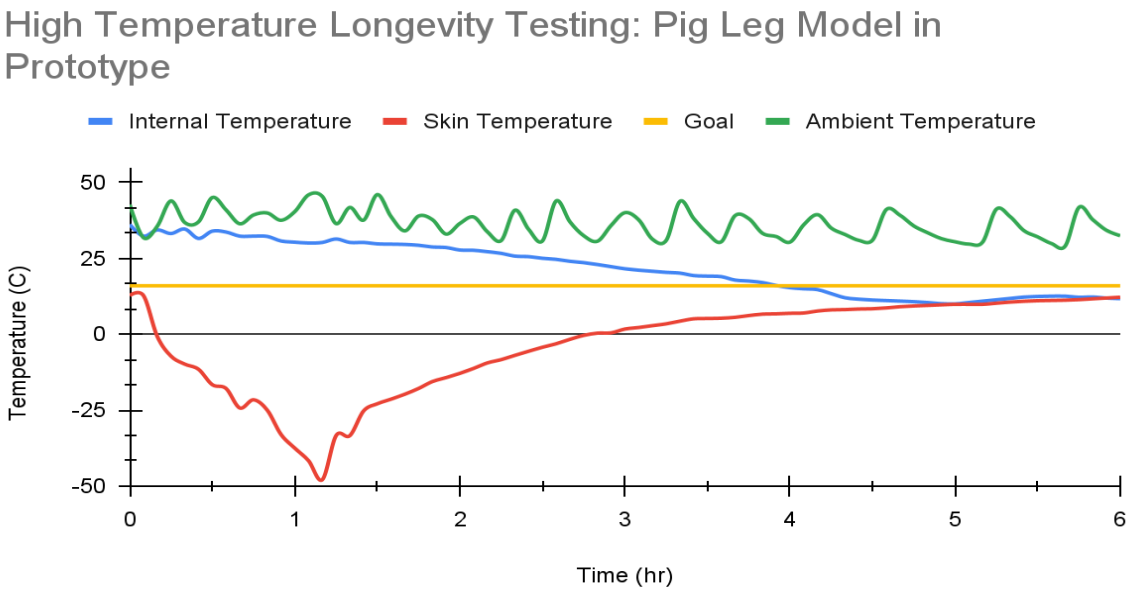


Figure 34. Temperature versus time data for pig leg with hip model for a six-hour period.

The internal temperature successfully reached the target temperature of 16 degrees Celsius and maintained this temperature throughout the whole time. This was done in an ambient temperature ranging from 35 degrees Celsius to 55 degrees Celsius. The temperature of the skin exhibits a significant drop around the one-hour mark, but no frost bite was observed on the skin at the conclusion of the test.

Chapter 6: Final Design and Validation

In a world of constant innovation, it is imperative to consider the impact that the device will have. Medical device innovations can not only reshape the field but also create massive impacts to the economy, society, ethics, manufacturability, politics, sustainability, and health and safety standards. The economic impacts of this device extend beyond the competition between devices and job markets. Simultaneously, it is essential to monitor the environmental footprint of the device. This is critical to properly addressing climate change. Society can be influenced as some devices can become catalysts for shifts in behavior, communications, and even cultural norms. Politically, this device could present new challenges in the government and international relations. Ethical concerns are always of importance in both the design, testing, manufacturing, and marketing of the device. Raising questions on equitable access, ethical testing measures, and more is essential to create a good, marketable device. To assess manufacturability of the device manufacturing location, process, and long-term impact on resources. Together, all these considerations allow for a multifaceted understanding of the device impact.

6.1: Economics

The current solution of an icebag is a cheaper alternative to our device. However, it is not the most effective solution. Our device will be economically beneficial to the patient than other prosthetic options which are significantly more expensive. Our device uses readily available materials which makes the cost of manufacture and production less expensive than the alternative solutions. The patient will have to pay for the reattachment surgery and any post-surgical rehabilitation recovery which will be determined by the healthcare professionals.

6.2 Environmental Impact

The environmental impact of the device manufacturing is low. The device requires a minimal number of materials and most of them do not produce an immense amount of CO_2 when manufactured. However, our device is not the most sustainable since the entire device cannot be reused. However, if hospitals sterilize the device and plan on reusing it then it will be more sustainable. The biohazardous materials from the limb will be disposed of properly and should not affect the general population. The device will also be properly disposed of by the hospital and should not affect the surrounding population. The only element that cannot be recycled is neoprene which is toxic to the environment. If the device is being implemented to other parts of the world, then the CO_2 produced by the aircraft will be considered when it is being exported from the US.

6.3 Societal Influence

This device was designed to be used solely by trained medical professionals. This could include emergency medical technicians (EMTs), trauma surgeons, or other personnel involved in high-risk medical situations. Although the goal was to make the device low-cost, external economic factors could impact the overall accessibility of this device and in lower-income areas. Some materials such as a liquid

CO_2 tank may be difficult to acquire and refill with limited financial resources. Furthermore, areas with scarce resources may not be able to receive the required training for proper use.

6.4 Political Ramifications

Although this device would be as helpful nationally as it is domestically, there are various other limiting factors in a foreign country's ability to use it. The primary concern would be access to all necessary materials, which may cause concerns with regulations across other borders. For example, the device is heavily reliant on liquid CO_2 tanks which may require importing. Lastly, if the device were to be used to transport between country borders, there are several health and safety regulations as well as origin of materials used. It is recognized that some of the materials used in this device were required to be imported to some capacity.

6.5 Ethical Concerns

Compared to what is currently the only solution of preserving partially and fully severed limbs (bag of ice water), the device aims to improve the standard. While ice water may slightly increase the time that the limb can be safely transported, it does not guarantee the limb will be cooled to the target temperature of 16-18 degrees Celsius. Our design aims to provide sufficient cooling for a long period of time to enable high limb preservation rates that may result in successful reattachment surgery. By enabling a greater chance of reattachment success, the direct beneficiary (person experiencing traumatic amputation) seeks a great quality of life. Quality of life is a direct effect of the device since preserving the limb long enough to avoid tissue necrosis will significantly decrease the need to experience full amputation. Through our device, beneficiaries may avoid multiple surgeries and other medical devices, such as prosthetics and other limb replacement technology. By avoiding full amputation, users can maintain their limb with minimal further damage.

6.6 Health and Safety Issues

With any reward comes risk. While the device provides great opportunity to avoid full amputations and the need of other medical aiding devices, there may be risk of further injury if not handled/employed properly. Specifically, due to the severe nature of the injury, handling of the limb must be thoroughly considered by EMS and other medical personnel using the device. Without thought, the limb or other surrounding parts of the body may be further injured. This can occur from mishandling—tugging and pulling—, misplacement, and misuse of the device. Despite the device's risks regarding further limb injury, safety concerns arise with the liquid CO2. Due to the nature of carbon dioxide, the liquid quickly turns to gas when exposed to ambient pressures and temperatures, with a portion of that gas then turning to a solid CO_2, known as dry ice. With the extreme low temperatures of dry ice, it is important to monitor the snow and its contact with the limb as frost bite may occur, causing further injury to the tissue that is meant to be preserved. Another concern is working with the CO_2 in an enclosed space, such as an ambulance. This would potentially require an increase in CO_2 venting. With proper training and meeting design specifications, these concerns may be greatly reduced.

6.7 Manufacturability

This device was designed to be integrated into EMS vehicles both in the United States (US) and internationally in US military applications. All components of the device are manufactured in the US and thus follow the FDA regulations and standards. The materials used in this project were utilized due to their broad accessibility, affordability, and adaptability in manufacturing. All materials employed in this device are listed in the Bill of Materials which can be found in (PLACE APPENDIX HERE). There are four components of this device that must be manufactured: the outer sleeve, the inner sleeve, single-use barrier, and the CO2 tank. They are assembled by the EMS professional when the device is used. Overall, the manufacturability of this device is not a concern. However, it is subject to change as this project will be taken over by future teams.

6.8 Sustainability

To create a sustainable device, one must complete a comprehensive analysis of the device's life cycle including the design, product, use, and disposal. To enhance the device's sustainability several steps were taken. In material selection, reusable materials were prioritized to lower the environmental impact. In the end only a small part of the device is single-use, and it is also recyclable after sterilization. Thus, allowing the life cycle of the device to be extended. The device is also very energy efficient. It does not require any electricity to use the device, and very little to maintain and control the device's temperature. The device was also designed with durability in mind. By creating a very durable device from the design to the materials selected it can dramatically extend the lifespan on the device. In summary, a lot of effort has been put into making this device sustainable by limiting single-use components, using material that can be recyclable, reducing energy requirements, and creating a durable design.

Chapter 7: Discussion

7.0 Discussion

This chapter discusses the testing done for verification using a pig leg model. The pig leg was tested through the state of the art and our device. The state of the art is to put the limb in a bag and place it in an ice bath for reattachment. Our device converts liquid to dry CO2. Sublimation of the CO2 causes the cool air to circulate the device and cool the limb.

7.1 Dry Ice Testing Using a Pig Leg

This test was done to verify the efficacy of the device compared to the state of the art. The pig leg was incubated at 37℃ to resemble body temperature. The pig leg was placed into our device in a controlled environment where the temperature was around 95 °F or 35℃. The pig leg was blasted twelve times with liquid CO2 every 5 minutes until a target temperature of 18℃ was acquired. The test lasted for six hours, and the surface temperature of the pig, the internal temperature of the pig, and the ambient temperature of the surrounding area were recorded every five minutes. From the test, the pig's internal temperature reached the target temperature in a little over three and a half hours. The pig leg maintained the internal temperature for the remaining two and a half hours of the experiment. It took longer for the pig leg to cool than the Jello model because a pig leg is not homogenous. It needs to cool actual fat and muscle which is going to take more time than a homogenous Jello model.

7.2 State of the Art testing Using Pig Leg

To compare the results from our results to the state of the art, the pig leg was placed into a biohazard plastic bag and submerged in a tub of ice water. The state-of-the-art test was done in a controlled temperature environment, like that of the dry ice testing using the pig leg. The ambient, surface, and internal temperatures were recorded every five minutes for six hours. At the end of the six hours, the internal temperature of the pig was 30.5℃ or 86.5°F. It did not reach the target temperature at any point in the six hours. It can be concluded that this is because the ambient temperature brought the temperature of the ice water to the ambient temperature and therefore the pig was not able to cool, since there was no mechanism for cooling. This proves that the state of the art is not an effective method for cooling a limb, and our device has promising results compared to it.

7.3 Theoretical Calculations for Human Model

The amount of dry ice used for the longevity testing at high temperatures for the pig leg surrogate was used to calculate the amount of liquid dry ice would be required for a 200 lb. person. This was done by first using calculating how much dry ice was used for this experiment this was done by using the equations below:

time tank was open * experimental volumetric flow rate of dry ice =weight of solid CO2 dispensed
Weight of solid CO2 dispensed/the experimental liquid to solid CO2 conversion rat=weight of liquid CO2
Weight of surrogate limb / Weight of liquid CO2 = weight of liquid CO2required per lb of limb
Weight of human limb * weight of liquid CO2 required per lb of limb = weight of liquid CO2 required for human

Once these equations are calculated the weight of liquid CO2 required to cool any size human limb can be approximated. The maximum theoretical weight of liquid CO_2 is 6.7 lbs. This is also an

overestimate since the limb was cooled far below target temperature during the experiment. The amount of liquid CO_2 and solid CO_2 required for different limbs can be found in Table 10.

Table 10 The amount of Dry Ice Needed for Common Amputations

Amputation Type (calculations for 200 lb person)	**Solid Dry Ice (lbs.)**	**Liquid CO_2 Conversion (lbs.)**
Total Leg (Including hip)	1.8	6.7
Lower Leg (Knee and below)	0.73	2.7
Foot	0.17	0.63
Total Arm (Including shoulder)	0.60	2.2
Lower Arm (Elbow and below)	0.22	0.81
Hand	0.08	0.30

Ultimately, this shows that the weight of this device including the weight of liquid carbon dioxide would be reasonable and would meet the design specifications.

7.4 Limitations of testing

One limitation of this test was controlling the ambient temperature. Since the heater used to achieve the ambient temperature was not stable, the temperature in the hood would fluctuate between 30 and 50℃. This made it difficult to mimic a constant desert environment, however, the results are still valid since the ambient temperature was usually over body temperature. Another limitation during testing was getting an accurate read of the surface temperature. The thermistor did not adhere to the pig skin properly and therefore was reading the temperature of the air inside the device rather than the pig skin. This has caused the surface temperature to reach -50℃, which is way below the target temperature and a state of frostbite. However, this was not the temperature of the pig skin but rather the cool air from the sublimation of the dry ice. Even though these limitations, the pig leg was able to reach the target temperature of 18℃ and keep it at that temperature afterwards, proving that the device is an effective method to cool a limb for six hours at 18℃.

Chapter 8: Conclusions and Recommendations

After investigating alternative limb preservation and cooling methods, the use of liquid carbon dioxide through a siphon tank was deemed the most efficient method of preventing major tissue necrosis. Liquid CO_2 reduces the need for excess materials such as water, electricity, and expertise to use the device. The conversion from liquid CO_2 (stored in a siphon tank) to solid dry ice allows for maximum portability and accessibility and is designed for use in the context of major amputation patients. To best accommodate both partial and full limb amputations, a cylindrical device was designed to effectively distribute the dry ice along the entire limb while remaining lightweight and portable. The device proved to have strong insulative properties when using a vinyl outer shell layer, followed by a reflective polyethylene terephthalate sheet, and polychloroprene foam (neoprene) in combination. The device also proved to have maximum cooling efficiency when using a housing system for the dry ice to fill when deployed into the device. The housing system ultimately allowed for the dry ice to be dispersed along the entire limb, providing maximum 360-degree cooling as opposed to filling a chamber in only one section of the device.

Through longevity testing with a surrogate limb (Jello model) the device's durability was proven successful in cooling the limb in an hour. This demonstrates the design and insulation built into the device were effective producing an ideal environment for the partially or fully amputated limb to rest in to maximize preservation and minimize tissue necrosis. Given the success of the longevity testing with the surrogate limb, the pig leg (including hip) was then tested to provide insightful data that would mimic a non-homogenous, robust, limb. The data proved the device to remain effective in cooling the limb, although it took about 3.5 hours to reach the target temperature. Despite taking longer to cool the limb thoroughly to the target temperature, it can be assumed that many layers of the limb cooled to the target temperature before the most internal location (where the temperature probe collected data). This promises that much of the tissue in the limb was able to cool fast enough to minimize necrosis and maximize the potential for successful reattachment surgery.

The device proved to be effective in housing the solid CO_2 and ensuring an efficient cooling system to the limb, yet it required ample attention for the first hour during the blasting cycles. To improve this, future work is recommended to design an automated system that controls the release of the CO_2 through the valve, allowing the responder to open the valve to the tank once the patient is comfortable in the device and let the system expel the dry ice in the blast intervals on its own. By automating the CO_2 release, it frees the medical personnel to work on maintaining comfort and addressing the patients' needs as they emerge. The blasting cycle being automated will also minimize error in missing a blast cycle or leaving the valve open for too long or too little time, ultimately affecting the cooling efficiency of the device. There should be communication between the chamber and skin temperature and the control system to alert it to cease release and notify the responding personnel if any concerning circumstances arise (i.e. low temperatures on skin, excessive warming or cooling in the chamber, faster/slower cooling than anticipated, etc.). In addition to automating and controlling the release system, it is recommended to have a pressure release automation system that acts as a secondary pressure release to the valve built into the device. This will ensure the patients' comfort while also minimizing unnecessary pressure built up in the device during the blasting system, given that a portion of what is expelled from the tank is gaseous CO_2.

The insulation of the device is crucial to its effectiveness but must also be considered in parallel with other design specifications such as size and weight. Although the combination of materials tested proved to be productive, the overall size and weight of the device can always be improved. It is recommended that different variations of the materials used are tested. For example, the thickness of the

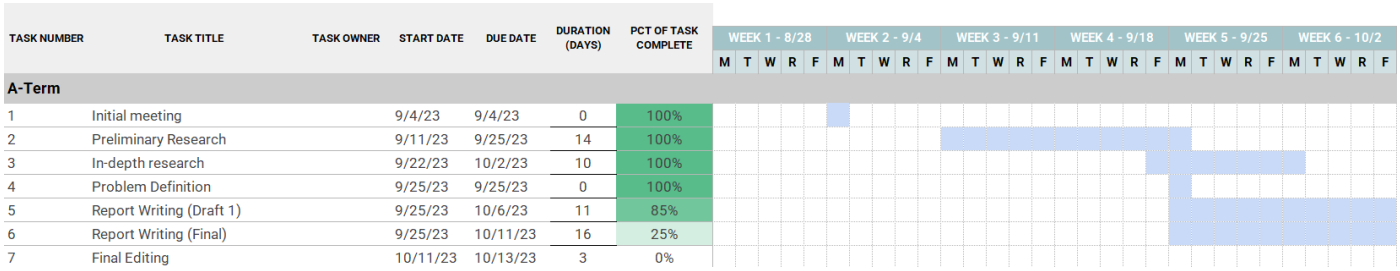
polychloroprene foam (neoprene) used was about 0.25 inches thick. This may be able to be reduced while keeping the same or similar insulative properties. The key will be finding the best balance of insulation and size/weight of the device and finding what the smallest amount of material needed is while retaining high insulation yield. This may be found within the materials or even the shape of the device being improved.

Lastly, it is recommended that frostbite and its causes be further investigated. Currently, very little solid research can prove an exact numerical value that indicates frostbite on the skin's surface with solid and gaseous CO_2. By understanding the trends of potential frostbite produced by CO_2, it will allow for a better expulsion rate and pattern into the device to minimize extenuating low temperatures on the skin's surface. Minimal direct contact between the skin and the dry ice would minimize the risk of dangerously low temperatures such as frostbite. This allows the system to work effectively without damaging the surface tissues.

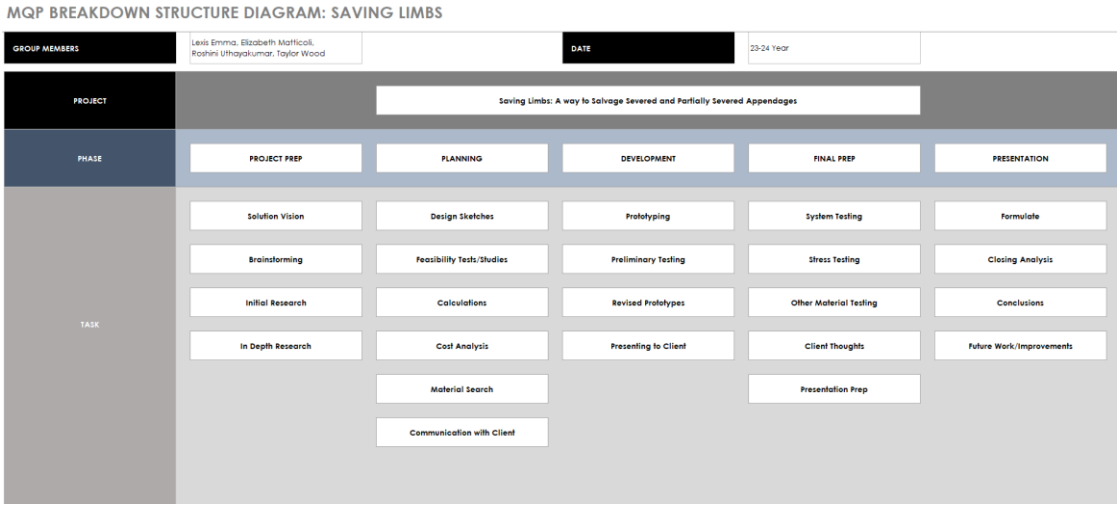
Appendix A
Project Management Methods

Gantt Chart Example:

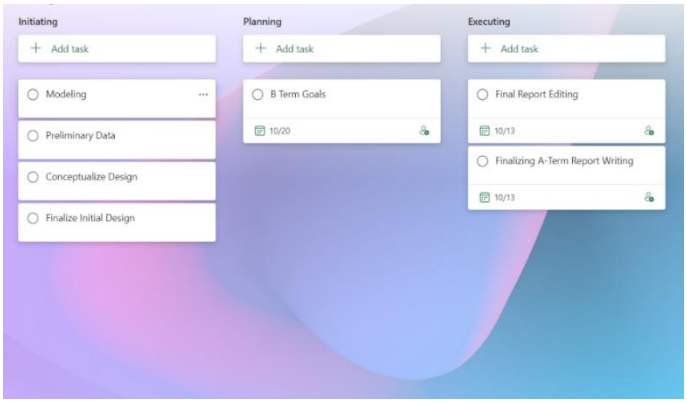
TASK NUMBER	TASK TITLE	TASK OWNER	START DATE	DUE DATE	DURATION (DAYS)	PCT OF TASK COMPLETE
A-Term						
1	Initial meeting		9/4/23	9/4/23	0	100%
2	Preliminary Research		9/11/23	9/25/23	14	100%
3	In-depth research		9/22/23	10/2/23	10	100%
4	Problem Definition		9/25/23	9/25/23	0	100%
5	Report Writing (Draft 1)		9/25/23	10/6/23	11	85%
6	Report Writing (Final)		9/25/23	10/11/23	16	25%
7	Final Editing		10/11/23	10/13/23	3	0%

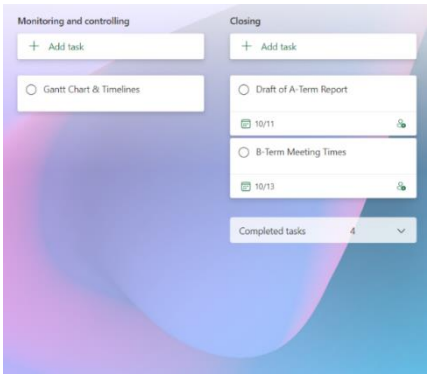


Work Breakdown Structure:



Planner:





Appendix B

Standard Operating Procedure

General:

Facility: WPI Laboratories

Project Director: Raymond Page

Scope: Testing Medical Device for Saving Limbs

Last Revision: 12/12/2023

Procedures:

I. TEMPERATURE PROBE CALIBRATION TESTING

Purpose:

The purpose of temperature probe calibration is to ensure the accuracy and reliability of temperature measurements, thereby maintaining the integrity of processes, experiments, and regulatory compliance.

Scope:

Temperature probe calibration is crucial for ensuring the accuracy of experimental conditions, maintaining the integrity of research data, and validating the performance of temperature-sensitive equipment such as incubators, ovens, and refrigerators.

Procedure:

1.	Set up two water baths: a. Fill two beakers up with water b. Place one beaker on a hot plate c. Place ice in second beaker	
2.	Inspect the temperature probe for any visible damage or signs of wear. Also ensure that the temperature probe is clean and free of any contaminants.	
3.	Allow the three water baths to stabilize temperature using reference thermometers in the calibration baths.	
4.	Place the temperature probes being tested into each water bath. Record the values of each temperature probe. Repeat for three trials.	
5.	Complete calculations using google sheet: here	

II. CO2 TANK LIQUID TO SOLID CONVERSION TESTING

Purpose:

The purpose of calculating the amount of dry ice snow created from liquid carbon dioxide (CO2) is to determine the quantity and distribution of dry ice particles produced during the sublimation process. Thus, in future tests the correct pressure and time can be calculated to ensure the correct amount of dry ice snow is created.

Scope:

The scope of calculating the conversion from liquid carbon dioxide to dry ice snow includes quantifying the volume and distribution of dry ice particles generated during the sublimation process, with applications of creating a specialized cooling system. This encompasses understanding the efficiency and effectiveness of the dry ice production process.

Definitions:

PPE: Personal protective equipment

Procedure:

1.	Ensure safety measures are in place: a. Proper PPE is worn (safety glasses, lab coat, gloves) b. Area is well ventilated c. Another person is present in the lab d. CO2 handling equipment (safe gloves)
2.	Measure the weight of the container and record the value.
3.	Gradually release the liquid CO2 into a covered bin. Start timer as soon as the release valve is opened. Keep the valve open for 30 seconds and then shut the valve. Record: a. actual time the valve was opened (s) b. the weight of a bin with dry ice snow in it (grams)
4.	Repeat 3 times for each time interval a. 10 seconds b. 15 seconds c. 20 seconds d. 30 seconds
5.	Repeat 3 times for each Trial.

6.	Complete calculations using google sheet: here

III. INITIAL MATERIAL TESTING: Outer Layer

Purpose:

The purpose of testing the outer layer of the device is to ensure thermal insulation and minimize heat transfer between the interior and exterior environments. This outer layer helps maintain the desired temperature of the limb stored inside, keeping it cool for an extended period and thus decreasing the amount of tissue necrosis that occurs.

Scope:

The scope of testing for the outer layer material includes assessing thermal insulation, heat transfer resistance, and the ability to maintain a consistent temperature to prevent tissue necrosis. The tests aim to ensure the durability and compliance of the outer layer with medical device regulations, providing realistic simulations to mitigate risks associated with the storage of limb transplants.

Procedure:

1.	Record the initial temperature inside the outer layer “bag”.	
2.	Place a bag of ice water inside the “bag”. Insert a temperature probe into the bag making sure it will not leak.	
3.	Close the “bag” using the drawstring mechanism. Record the starting temperature.	
4.	Record temperature every minute.	
5.	Repeat for three trials for every material being tested	
6.	Complete calculations using google sheet: here	

IV. INITIAL MATERIAL TESTING: Contact Layer

Purpose:

The purpose of testing the contact layer is to observe the flexibility and resistance to freezing at low temperatures.

Scope:

The scope of material testing for the contact layer involves assessing its ability to prevent frostbite by providing effective insulation and evaluating its thermal conductivity to ensure efficient limb cooling.

This encompasses the examination of material properties and performance characteristics relevant to both protection and cooling requirements.

Procedure:

1.	Hydrate Orbeez for 15 minutes and 50 minutes. Place them into two seperate vacuum sealable bags and vacuum seal it on moist setting. Do the same with the Up and Up gel beads.
2.	Place all three gel packs in the freezer for 24 hours.
3.	Observe the flexibility, stiffness, texture, etc. of all the gel packs. Record observations and take images.
4.	Allow packs to thaw. Observe the gel packs after they thaw. Record observations and take images.

V. STATE OF THE ART TESTING

Purpose:

The purpose of testing a state-of-the-art device is to create a baseline when assessing performance, functionality, and features, identifying areas for improvement in our device's design and ensuring its competitiveness in the market. This comparative testing is crucial for refining and optimizing our device to meet or exceed industry standards and user expectations.

Scope:

The scope of this experiment is to test the cooling from our device's design against the traditional method of using ice in a bag by assessing the efficiency, precision, and safety of both cooling approaches. This includes evaluating temperature control, preservation effectiveness, and potential benefits or drawbacks in order to inform the optimization and potential adoption of the CO2-based cooling method for amputated limb storage.

Procedure:

1.	Cut a hole into the cap of a 2 L bottle. Insert the calibrated thermistor in the center of the bottle. Ensure that the holes around the wires are leak proof.
2.	Fill bottle with water and appropriate amount of Jello/gelatin
3.	Place the water bottle in the oven, remove once the center probe reaches 37°C.

4.	Secure probes to Arduino and ensure temperatures are being recorded
5.	Place a bag full of water and ice on the water bottle.
6.	Record the room temperature, the internal temperatures of the bottle every minute until the device reaches 16 C.
7.	Complete calculations using google sheet: here

V. DEVICE DESIGN TESTING

Purpose:

This procedure aims to evaluate multiple device designs to identify the most effective solution for a specific application. By comparing performance across various criteria, it seeks to inform decision-making and facilitate the selection of the optimal design.

Scope:

This procedure involves testing multiple device designs to determine their cooling efficiency, measured in degrees Celsius per minute (°C/min). By comparing cooling rates under controlled conditions, the goal is to identify the most effective design for rapid cooling applications.

Procedure:

1.	Cut a hole into the cap of a 2 L bottle. Insert the calibrated thermistor in the center of the bottle and the surface of the bottle. Ensure that the holes around the wires are leak proof.
2.	Fill bottle with water and appropriate amount of Jello/gelatin
3.	Place the water bottle in the oven, remove once the center probe reaches 37°C.
4.	Secure probes to Arduino and ensure temperatures are being recorded
5.	Place the Jello into the device currently being tested. Blast the CO2 every 5 minutes for 10 seconds. Record the temperature of the surface temperature and the internal temperature every minute.
6.	Complete calculations using google sheet: here

VI. LONGEVITY TESTING OF FINAL DEVICE

Purpose:

The purpose of this experiment is to assess the effectiveness of the device in maintaining a consistent and sufficiently low temperature to enable successful limb replantation procedures. Limb replantation surgeries require a preserved limb with minimal tissue damage, and maintaining optimal cooling conditions is critical for the viability of the replanted limb. This experiment aims to evaluate whether the device can sustain cooling for a duration of 6 hours thereby ensuring the potential success of such procedures.

Scope:

This experiment aims to test the device's ability to maintain limb cooling for 6 hours, crucial for successful replantation surgeries. Using temperature sensors and simulated surgical conditions, the team will assess its effectiveness and suitability for clinical use.

Procedure:

1.	Cut a hole into the cap of a 2 L bottle. Insert the calibrated thermistor in the center of the bottle and the surface of the bottle. Ensure that the holes around the wires are leak proof.
2.	Fill bottle with water and appropriate amount of Jello/gelatin
3.	Place the water bottle in the oven, remove once the center probe reaches 37°C.
4.	Secure probes to Arduino and ensure temperatures are being recorded
5.	Place the Jello into the device currently being tested. Blast the CO2 every 5 minutes for 10 seconds until the internal temperature reaches 16 C. Record the temperature of the surface temperature and the internal temperature every five minute for 6 hours.
6.	Complete calculations using google sheet: here

Appendix C

Arduino Code for Thermistors

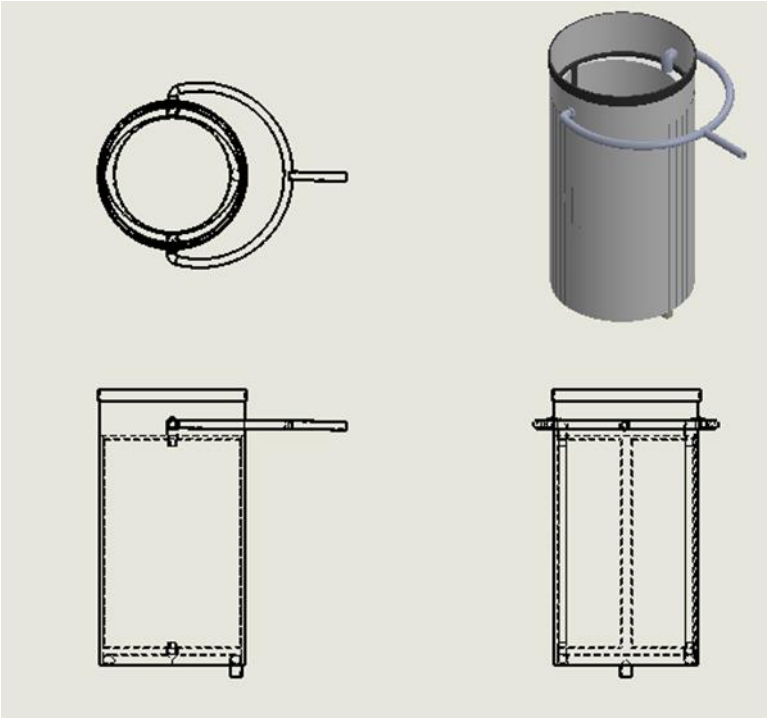
```
int ThermistorPin_1 = 0;
int ThermistorPin_2 = 1;
int ThermistorPin_3 = 2;
int ThermistorPin_4 = 3;
int ThermistorPin_5 = 4;
int ThermistorPin_6 =5;

int Vo_1;
int Vo_2;
int Vo_3;
int Vo_4;
int Vo_5;
int Vo_6;

float R1 = 10000;
float logR2_1, R2_1, T_1, Tc_1, Tf_1;
float logR2_2, R2_2, T_2, Tc_2, Tf_2;
float logR2_3, R2_3, T_3, Tc_3, Tf_3;
float logR2_4, R2_4, T_4, Tc_4, Tf_4;
float logR2_5, R2_5, T_5, Tc_5, Tf_5;
float logR2_6, R2_6, T_6, Tc_6, Tf_6;

float c1 = 1.009249522e-03, c2 = 2.378405444e-04, c3 = 2.019202697e-07;

void setup() {
Serial.begin(9600);
}

void loop() {

  Vo_1 = analogRead(ThermistorPin_1);
  Vo_2 = analogRead(ThermistorPin_2)
  Vo_3 = analogRead(ThermistorPin_3);
  Vo_4 = analogRead(ThermistorPin_4);
  Vo_5 = analogRead(ThermistorPin_5);
  Vo_6 = analogRead(ThermistorPin_6);



  R2_1 = R1_1 * (1023.0 / (float)Vo_1 - 1.0);
  logR2_1 = log(R2_1);
  T_1 = (1.0 / (c1 + c2*logR2_1 + c3*logR2_1*logR2_1*logR2_1));
  Tc_1 = T_1 - 273.15;
  Tf_1 = (Tc_1 * 9.0)/ 5.0 + 32.0;

  R2_2 = R1_2 * (1023.0 / (float)Vo_2 - 1.0);
  logR2_2 = log(R2_2);
```

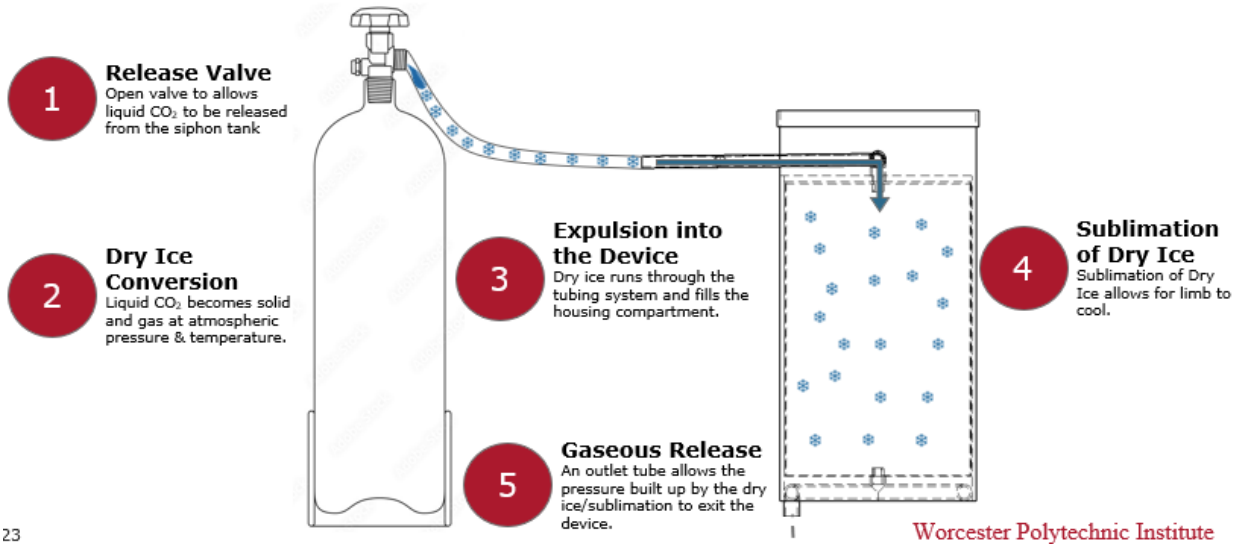
```
T_2 = (1.0 / (c1 + c2*logR2_2 + c3*logR2_2*logR2_2*logR2_2));
Tc_2 = T_2 - 273.15;
Tf_2 = (Tc_2 * 9.0)/ 5.0 + 32.0;

R2_3 = R1_3 * (1023.0 / (float)Vo_3 - 1.0);
logR2_3 = log(R2_3);
T_3 = (1.0 / (c1 + c2*logR2_3 + c3*logR2_3*logR2_3*logR2_3));
Tc_3 = T_3 - 273.15;
Tf_3 = (Tc_3 * 9.0)/ 5.0 + 32.0;

R2_4 = R1_4 * (1023.0 / (float)Vo_4 - 1.0);
logR2_4 = log(R2_4);
T_4 = (1.0 / (c1 + c2*logR2_4 + c3*logR2_4*logR2_4*logR2_4));
Tc_4 = T_4 - 273.15;
Tf_4 = (Tc_4 * 9.0)/ 5.0 + 32.0;

R2_5 = R1_5 * (1023.0 / (float)Vo_5 - 1.0);
logR2_5 = log(R2_5);
T_5 = (1.0 / (c1 + c2*logR2_5 + c3*logR2_5*logR2_5*logR2_5));
Tc_5 = T_5 - 273.15;
Tf_5 = (Tc_5 * 9.0)/ 5.0 + 32.0;

R2_6 = R1_6 * (1023.0 / (float)Vo_6 - 1.0);
logR2_6 = log(R2_6);
T_6 = (1.0 / (c1 + c2*logR2_6 + c3*logR2_6*logR2_6*logR2_6));
Tc_6 = T_6 - 273.15;
Tf_6 = (Tc_6 * 9.0)/ 5.0 + 32.0;

Serial.print("Temperature Probe 1: ");
Serial.print(Tf_1);
Serial.print(" F; ");
Serial.print(Tc_1);
Serial.println(" C");

Serial.print("Temperature Probe 2: ");
Serial.print(Tf_2);
Serial.print(" F; ");
Serial.print(Tc_2);
Serial.println(" C");

Serial.print("Temperature Probe 3: ");
Serial.print(Tf_3);
Serial.print(" F; ");
Serial.print(Tc_3);
Serial.println(" C");


Serial.print("Temperature Probe 4: ");
Serial.print(Tf_4);
Serial.print(" F; ");
```

```
  Serial.print(Tc_4);
  Serial.println(" C");

  Serial.print("Temperature Probe 5: ");
  Serial.print(Tf_5);
  Serial.print(" F; ");
  Serial.print(Tc_5);
  Serial.println(" C");

  Serial.print("Temperature Probe 6: ");
  Serial.print(Tf_6);
  Serial.print(" F; ");
  Serial.print(Tc_6);
  Serial.println(" C");



  delay(60000);
}
```

Appendix D

Process Flow and SolidWorks Drawing





Appendix E

Final Longevity Testing Procedure

Purpose:

This experiment assesses the device's effectiveness in maintaining a consistent and sufficiently low temperature to enable successful limb replantation procedures. Limb replantation surgeries require a preserved limb with minimal tissue damage, and maintaining optimal cooling conditions is critical for the viability of the replanted limb. This experiment aims to evaluate whether the device can sustain cooling for six hours, ensuring the potential success of such procedures.

Scope:

This experiment aims to test the device’s ability to maintain limb cooling for 6 hours, crucial for successful replantation surgeries. Using temperature sensors and simulated surgical conditions, the team will assess its effectiveness and suitability for clinical use.

Procedure:

1.	All participating members put on proper PPE (lab coat, surgical gloves, eyeglasses).
2.	The model sample is brought to 37 degrees Celsius using an incubator.
3.	The heater is placed under the fume hood and turned on to 35 degrees Celsius.
4.	Once target temperature is reached internally, remove the model sample and attach the thermistors: one in the center and one on the surface. A third thermistor is placed in the center of the hood.
5.	The model is placed in the device, sealed, and moved under the hood. Use caution when placing the sample into the device so as to not move the internal bag system.
6.	All necessary tubing connections are completed, including the CO_2 tank tubing and the exit tubing.

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