



# WPI

Oxygen Topical Treatment for Wound Care

A Major Qualifying Project Report

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of

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by

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## Contents

Acknowledgments	5
Abstract	6
Authorship Page	7
Table of Figures	8
Chapter 1 Introduction	9
Chapter 2 Background and Literature Review	11
2.1: Wound Healing	11
2.2: The Process of Wound Healing	12
2.3: Chloroplast Functions	12
2.4: Current Treatments & Research	13
2.4.1: Wound Dressing (Hydrogel)	13
2.4.2: Hyperbaric Oxygen Therapy (HBOT)	14
2.4.3: Oxygen and Electrotherapy	14
2.4.4: Vacuum-assisted Closure Therapy (VAC)	15
2.5: Disadvantages of Current Treatments	15
2.5.1: Hydrogel	15
2.5.2: HBOT	16
2.5.3: Vacuum-assisted Closure Therapy	16
Chapter 3 Project Strategy	17
3.1 Initial Client Statement	17
3.2 Objectives	17
3.2.1 Safety	18
3.2.2 Delivery of Oxygen	19
3.2.3 Consumers	19
3.2.4 Quality Control	19
3.3 Functions	20
3.3.1 Produce Oxygen	20
3.3.2 Ease of Use	21
3.3.3 Barrier	21
3.3.4 Flexibility	21
3.4 Standards	21

3.4.1 Dermal Safety Testing	22
3.4.2 Hydrogels and Hyperbaric Oxygen Therapy	25
3.4.3 Labeling and Packaging	26
3.4.4 Quality	27
3.4.5 Storage	28
3.5 Constraints	28
3.5.1 Size of Application	29
3.5.2 Cost	29
3.5.3 Safety	30
3.5.4 Comparisons to the Market	30
3.6 Specifications	30
3.6.1 Storage	31
3.6.2 Resistance to Elements	31
3.6.3 Oxygen Production	32
3.7 Revised Client Statement	32
3.8 Management Approach	32
Chapter 4 Design Process	34
4. 1 Needs Analysis	34
4.1.1 Pairwise Comparison	34
4.1.2 Ranking the Requirements	36
4.2 Conceptual Designs	38
4.3 Alternative Designs:	39
4.3.1 Vaseline infused gel	40
4.3.2 Hand sanitizer gel	40
4.3.3 Glycerol Hydrogel	40
4.3.4 Band-aid infused with a layer of gel	40
4.4. Final Design Selection:	41
Chapter 5 Design Verification	42
5.1 Initial Preparing	42
5.2 Choosing the Material	43
5.2.1 Vaseline	43
5.2.2 Hand Sanitizer	45

5.2.3 Glycerol	45
5.3 Testing	46
5.3.1 Hill Reaction	46
Chapter 6 Final Design and Validation	48
6.1 Final Design	48
6.1.1 Isolating Chloroplasts	48
6.1.2 Preparing the Solution	49
6.2 Impact Analysis	49
6.2.1 Economics	49
6.2.2 Environmental Impact	50
6.2.3 Societal Influence	51
6.2.4 Political Ramifications	51
6.2.5 Ethical Concerns	52
6.2.6 Health and Safety Issues	52
6.2.7 Manufacturability	53
6.2.8 Sustainability	53
Chapter 7 Discussion	54
Chapter 8 Conclusion	57
8.1 Future Work	57
8.2 Recommendations	58
8.2.1 Disinfection and Sterilization	58
References	60
Appendices	62
Appendix A: B-term Gantt Chart	62
Appendix B: Sterilization vs Disinfection	63
Appendix C: Microscope Images Hand Sanitizer (40x)	64
Appendix D: Chloroplast Solution (20x)	66
Appendix E: Materials Needed	68

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## **Abstract**

According to a study, in 2018, it was estimated that the global market for topical products valued at over 9 billion dollars in 2014, which is only increasing. The load of treating these wounds is growing rapidly due to many different issues. These issues may include health care costs, and aging population. Another issue that has been growing over the years especially in the United States is a rise in the number of patients with diabetes and obesity. Currently there are some treatments that can deal with wound healing which include antibiotics, ointments, band-aids, etc. The basic steps of wound healing are homeostasis, inflammation, growth and rebuilding and strengthening. Within minutes or even seconds, the blood cells start to clump together and create a clot. This project aims to design and produce a new and renovated hydrogel. This new hydrogel will provide an additional amount of oxygen to the affected wounded area. The new product was tested for whether oxygen was still being produced once the batch was completed.

## Authorship Page

Section		Name	Section		Name
1	Introduction	J. P.	3.7	Revised Client Statement	J. P.
2	Background and Literature Review	J. P.	3.8	Management Approach	A.N.
2.1	Wound Healing	A.N.	4	Design Process	J. P.
2.2	The Process of Wound Healing	A.N.	4.1	Needs Analysis	J. P.
2.3	Chloroplast Functions	J. P.	4.1.1	Pairwise Comparison	J. P.
2.4	Current Treatments and Research	A.N.	4.1.2	Ranking the Requirements	J. P.
2.4.1	Wound Dressing (Hydrogel)	J. P.	4.2	Conceptual Designs	J. P.
2.4.2	Hyperbaric Oxygen Therapy	J. P.	4.3	Alternative Designs	J. P.
2.4.3	Oxygen and Electrotherapy	A.N.	4.3.1	Vaseline Infused Gel	J. P.
2.4.4	Vacuum-assisted Closure Therapy	J. P.	4.3.2	Hand Sanitizer Gel	J. P.
2.5	Disadvantages of Current Treatments	A.N.	4.3.3	Glycerol Hydrogel	J. P.
2.5.1	Hydrogel	ALL	4.3.4	Band-aid infused with Gel	J. P.
2.5.2	HBOT	J. P.	5	Design Verification	J. P.
2.5.3	Vacuum-assisted Closure Therapy	A.N.	5.1	Initial Preparing	J. P.
3	Project Strategy	J. P.	5.2	Choosing the Material	J. P.
3.1	Initial Client Statement	J. P.	5.2.1	Vaseline	J. P.
3.2	Objectives	ALL	5.2.2	Hand Sanitizer	J. P.
3.2.1	Safety	J. P.	5.2.3	Glycerol	J. P.
3.2.2	Delivery of Oxygen	J. P.	5.3	Testing	J. P.
3.2.3	Consumers	ALL	5.3.1	Hill Reaction	J. P.
3.2.4	Quality Control	ALL	6	Final Design and Validation	J. P.
3.3	Functions	A.N.	6.1	Final Design	J. P.
3.3.1	Produce Oxygen	ALL	6.1.1	Isolating Chloroplast	J. P.
3.3.2	Ease of Use	A.N.	6.1.2	Preparing the Solution	J. P.
3.3.3	Barrier	A.N.	6.2	Impact Analysis	J. P.
3.3.4	Flexibility	A.N.	6.2.1	Economics	J. P.
3.4	Standards	J. P.	6.2.2	Environmental Impact	J. P.
3.4.1	Dermal Safety Testing	J. P.	6.2.3	Societal Influence	J. P.
3.4.2	Hydrogels and Hyperbaric Oxygen Therapy	A.N.	6.2.4	Political Ramification	J. P.
3.4.3	Labeling and Packaging	J. P.	6.2.5	Ethical Concerns	J. P.
3.4.4	Quality	J. P.	6.2.6	Health and Safety Issues	J. P.
3.4.5	Storage	J. P.	6.2.7	Manufacturability	J. P.
3.5	Constraints	A.N.	6.2.8	Sustainability	J. P.
3.5.1	Size of Applications	A.N.	7	Discussion	A.N.
3.5.2	Cost	A.N.	8	Conclusion	A.N.
3.5.3	Safety	ALL	8.1	Future Work	J. P.
3.5.4	Comparisons to the Market	A.N.	8.2	Recommendations	A.N.
3.6	Specifications	A.N.	8.2.1	Disinfection and Sterilization	J. P.
3.6.1	Storage	A.N.			
3.6.2	Resistance to Elements	A.N.			
3.6.3	Oxygen Production	A.N.			

## Table of Figures

Figure 1-1 Steps of Wound Healing (Wijesinghe et al; 2014).....	9
Figure 4-1 Basic Concept Map .....	38
Figure 4-2 Detailed Concept Map.....	39
Figure 5-1 Initial Preparation Materials.....	42
Figure 5-2 Chloroplast Solution.....	43
Figure 5-3 Chloroplast Solution with Vaseline .....	44
Figure 5-4 Chloroplast Solution with Heated Vaseline .....	44
Figure 5-5 Chloroplasts with Hand Sanitizer.....	45
Figure 5-6 Hill Reaction with Hand Sanitizer .....	46
Figure 5-7 Hill Reaction Under Ambient Light.....	46
Figure 5-8 Hill Reaction Under a Lamp: Left Initial at 0 minutes, Right After 7 minutes .....	47
Figure 6-1 Unit sales of Brand Names (Shahbandeh, 2019) .....	50
Figure 6-2 Global Wound Care Market Share (Wound Care Size, 2019).....	52
Figure 8-1 Ranking the Bacteria Killed (CDC, 2016) .....	59



## Chapter 1 Introduction

The overall goal of this project was to design a new topical treatment, which will provide an addition of 5%-10% oxygen to the affected wounded area. This treatment will be safe for the patient to apply at the convenience of their home. This product will be able to be stored for at least six months. The topical treatment product will be consistent with the initial batch. This product will ensure a broad range of potential consumers. In the world there are about 6.5 million patients dealing with chronic wounds (Sen, et al; 2009). This has been an increasing problem for patients around the world. The number has been increasing due to the amount of health problems being increased such as diabetes, and obesity. These health problems make it harder for wounds to heal due to their underlying conditions.

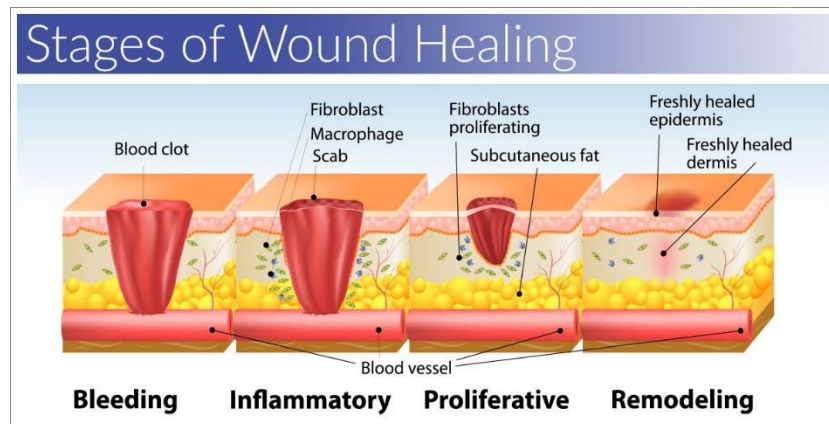


Figure 1-1 Steps of Wound Healing (Wijesinghe et al; 2014)

There are four major steps which involve wound healing which are bleeding, inflammatory, proliferative, and remodeling. The first step is to stop the bleeding which is usually begun by the blood cells starting to clump together and clot. The next step, inflammatory is when one the wound is closed by the clot the blood vessels which allow nutrients and oxygen to enter. Blood-borne oxygen is needed for healing (DeMuro, Fraser & Foley). During proliferative the blood cells,

oxygen-rich blood cells will help build new tissue. The final stage remodeling is once the wound begins to regain strength and finally repair itself. The topical products which are currently in the market are mostly used for wound healing and taking care of a wound. As of now the only product being used for wound healing that incorporates oxygen is a hyperbaric oxygen chamber. This is a major reason for conducting these experiments, in order to help patients, heal their wounds at a faster pace.

The following chapters in this report will discuss various factors leading to the final design of the product. They will also discuss how the product will have an impact on the global side of the market as well as on patients and people all around the world. The chapters will describe the clinical process of fabricating the oxygen producing hydrogel for wound healing, the project approach, and validating the design via different testing methods. The results from these tests described the correct ratio fabrication of the gel and chloroplast solution. Finally, the final design is analyzed based on the goals specified in the client statement. The team also provided recommendations and future works that can be considered.

## **Chapter 2 Background and Literature Review**

Background and literature research were conducted in order to familiarize with the scope of the project. Another reason was to understand the healing process of a wound and the effects of oxygen in them. The following sections give an overview of the process of wound healing, how chloroplasts functions and any current treatments being used.

### **2.1: Wound Healing**

When we sustain an injury such as a small cut or open wound, our bodies undergo a complex sequence of events to ensure that the wound is healed to the best of our abilities. Through the process of hemostasis, the blood cells start to clump together and clot which serves to both protect and close the wound area. Inflammation occurs where the blood vessels open to allow nutrients and oxygen into the wound for the healing process. Blood-borne oxygen is essential for healing. Inadequate blood supply, especially the lack of oxygen-rich blood, is one of the most crucial factors for interrupted wound healing. A wound not getting enough blood to deliver the essential nutrients and oxygen can take at least twice as long to heal properly. These are called chronic wounds that do not heal in an orderly set of stages. Wound healing can be taken for granted as it is the way our bodies attempt to revert to its original healthy state.

The start of the process begins with acute hypoxia and leads into oxygen recovery. Wound areas have a disrupted local vascular supply. The state of the wound also determines how effective the oxygen is when the wound is hypoxic, normoxia, or hyperoxia. Chronic hypoxia can impair the healing process as a result of not healing in an orderly set of stages.

## **2.2: The Process of Wound Healing**

The basic steps of wound healing are hemostasis, inflammation, growth & rebuilding and strengthening. Within minutes or even seconds, the blood cells start to clump together and create a clot. This protects the wound from further blood loss as well as creating the first barrier to preventing wound infection before further care is applied. In inflammation, the blood vessels deliver nutrients and oxygen to the affected area in the process of healing. The levels of oxygen are important in this stage as too much or too little impairs efficient healing. As the repair process continues, blood cells arrive to build new tissue. In the process of granulation, tissue and microscopic blood vessels form on the surface of the wound by growing from the base and fills in. Collagen is created and serves as a scaffold for repairs to the area as more tissue fills in the wound.

## **2.3: Chloroplast Functions**

Plants, as well as eukaryotic algae contain an organelle which helps them produce food. These organelles are known as chloroplasts, and they conduct this by a process known as photosynthesis. Photosynthesis is the process by which plants convert carbon dioxide and water into carbohydrates and oxygen by using the energy of the sun. Sunlight enters the chloroplasts, which then encounter the chlorophyll. There are different types of chlorophyll (a, b, c, d, and e) which each absorb energy from different wavelengths of the light. Chlorophyll a absorbs the energy from the violet-blue and reddish wavelengths. Once the chlorophyll traps the energy it will begin to split the water molecules into separate ions as well as free electrons. From these ions that were split the oxygen ion is released into the atmosphere while the hydrogen ion is carried to the stroma of the chloroplast. The remaining energy absorbed by the chlorophyll will be carried to the stroma, where the carbohydrates will be made. In the stroma it will combine the hydrogen ion, the

carbon dioxide which entered the leaf of the plants and it will be made into carbohydrates. Once this process is completed carbohydrates will be carried to the cells of the plant.

## **2.4: Current Treatments & Research**

Today's research and currently used methods of treating wounds are still expanding. Oxygen is a vital component in the process of wound healing but promoting higher levels of oxygen in the affected wound areas outside from the delivery of the necessary components from the blood vessels is the proof of principle that present-day research is still working on. Current known treatments today include a variety of applications and therapy which take advantage of adapting to work with the body. These methods are not only limited to providing 100% of pure oxygen but are also maintaining an optimum wound healing environment.

### **2.4.1: Wound Dressing (Hydrogel)**

Hydrogel dressings aids the body in protecting it from any infection and promotes efficient healing. The dressing provides a favorable healing environment by maintaining the wound warm and moist. This dressing is made up of about 90% water which is put on the gel. This gel is composed of hydrophilic polymers, which swell up they encounter water. The hydrogel provides moisture, which helps granulation, healing, as well as keeping the wound cool. This aids in alleviating the pain.

Hydrogels are available in two types of forms: sheets or amorphous gels. From these two types of forms there are three types of hydrogel: hydrogel sheets, amorphous hydrogels and impregnated hydrogels. Hydrogel sheets are made of polymeric cross-linked molecules which are capable of absorbing water. The backing of the sheet aids in regulating evaporation from the sheet

and always keeps the wound moist. Amorphous hydrogels are gel like and can be applied freely onto the surface. The gel enters the crevices of the wound regardless of the depth of the wound. Usually, after applying the gel it may be covered with a gauze to it the gel in place. Impregnated hydrogels are gels applied to a gauze, sponge strip or pad, which then cover the wound and are further covered by a secondary dressing to seal the wound.

#### **2.4.2: Hyperbaric Oxygen Therapy (HBOT)**

Hyperbaric oxygen therapy is a type of therapy which exposes the body to a 100% of pure oxygen. HBOT aids in many different forms, such as decompression sickness, serious infections, bubbles of air in the blood vessels and wounds that won't heal. This therapy involves breathing in pure oxygen in a pressurized room or a chamber. Inside the chamber, the air pressure is increased three times higher than the normal pressure. Increasing the pressure increases the amount of oxygen you can breathe in. Once the oxygen is traveling throughout the body, it stimulates the release of growth factors and stem cells.

#### **2.4.3: Oxygen and Electrotherapy**

There has been some research that demonstrates that if oxygen is administered, it can accelerate and sustain vessel growth. This phase is crucial in the start of wound healing as the local vessel blood supply must deliver the necessary materials to the affected area. Furthermore, there has been research conducted on the use of nanotechnology to create electrical pulses that induce mechanical displacements with body and muscle motions. This opens the path for more research to continue as it demonstrates the feasibility of electrical stimulations that are self-sustainable and biologically responsive.

#### **2.4.4: Vacuum-assisted Closure Therapy (VAC)**

Vacuum-assisted closure therapy (VAC) is a type of therapy in which it aids to heal wounds. A wound vacuum system has several parts. A foam or gauze dressing is put directly on the wound. An adhesive film covers and seals the dressing and wound. A drainage tube leads from under the adhesive film and connects to a portable vacuum pump. This pump removes air pressure over the wound. It may do this either constantly. Or it may do it in cycles. During the treatment, a device decreases air pressure on the wound. This can help the wound heal more quickly.

#### **2.5: Disadvantages of Current Treatments**

Despite current research development and methods of treatment used today, there still exist some disadvantages that make the application of oxygen in wound healing difficult. Primarily considering the method of application, current treatments still have room for improvement as the biological signals and functions of the human body must be considered in designing methods of treatment.

##### **2.5.1: Hydrogel**

Even though a hydrogel has many advantages to it, there are some disadvantages that may prevent anyone from using it. A disadvantage would be that a hydrogel cannot absorb large amounts of liquids. This makes the hydrogel unsuitable for a wound that is secreting a lot of fluid. Furthermore, the trait of having low mechanical strength puts the durability of the application into consideration. If it tears, fluids spreading outside the wound area would be an inconvenience for the patient.

### **2.5.2: HBOT**

Hyperbaric oxygen therapy is usually a safe procedure, but there are instances where some complications may occur. This treatment does carry some risks which may be both short-term and long-term. A short-term risk may be temporary myopia, which is caused by changes in the eye lenses. Due to increasing air pressure there are some risks associated with this. One of the risks is related to your ear, for example your eardrum may rupture as well as there can be leaking fluid. Another risk would be lung barotrauma which would cause the lung to be damaged or a lung collapsing. Due to the increase of oxygen there are also two major risks associated with this. One risk would be seizures which are caused by too much oxygen in the central nervous system. Another risk would be fire, which is due to the oxygen-rich atmosphere in the chamber.

### **2.5.3: Vacuum-assisted Closure Therapy**

The limitations of vacuum-assisted closure therapy are also plain in sight. Due to using a suction dressing, the device creates negative pressure on the wound. However, this machine also conducts noise and limits the patient's mobility. The wound dressing consists of a sponge applied on top of the wound. During the healing process, the granulation tissue and nerve endings that regenerate can grow into the sponge material. The process of removing and redressing the wound can induce little to significant pain as a result of removing the sponge.



## **Chapter 3 Project Strategy**

In this chapter the initial client statement was discussed. The objectives of the project were discussed as well as goals, functions, and standards. There are also different types of test that may be performed in order to determine the quality of the product. In this chapter the different competitions that are out there are also explained.

### **3.1 Initial Client Statement**

At the beginning of the project, the client and the team generated an initial client statement based on the main goal of this product. The main goal is to produce any topical product that will be able to give off oxygen to help the wound heal.

“Design a topical treatment to provide increased oxygen to the surface of an open wound.”

This initial statement has been improved by stating the goals or objectives that the product will need to comply with. Some the objectives that will be incorporated in this statement are listed in list #1 (section 3.2).

### **3.2 Objectives**

Identification of the objectives was the initial step in determining the roadmap for the technical aspect of the final design. An objective is a goal or when someone aims to achieve a desired result. These objectives are listed below in list #1. The team determined these objectives based on the client’s needs or wants. Another way of finalizing the objective list was by using Information from the research conducted for chapter 2. It has helped decrease the long list of

objectives and categorize them into these specific 5 objectives. The final design should be safe for the consumers to use, deliver oxygen, provide a range of potential consumers, and ensure quality control.

1. Safety
2. Delivery of Oxygen
3. Consumers
4. Quality Control

List #1: Main topics for objectives

### **3.2.1 Safety**

Safety has been one of the major objectives that needs to be complied with for this project. The federal government also has three agencies who are responsible for ensuring consumer safety. These three agencies are the Food and Drug Administration (FDA), the Consumer Product Safety Commission (CPSC) and the Environmental Protection Agency (EPA) (Why is Product Safety Needed). Some of the toxicity or characteristics that classify a product unsafe for a consumer to use may include but are not limited to products that cause any irritation, inflammation, or further infection to the affected wounded area (Grimes & Lyssikatos, 2018). The wound dressing should be safe for the user, complying with FDA, ISO, and ASTM standards and regulations. Some of the characteristics that we may consider for this product is for it to not cause any irritation, rash or any infection to the wounded area.

### **3.2.2 Delivery of Oxygen**

The delivery of oxygen is an important objective for this product since oxygen is the main “ingredient” involved in the composition of the topical product. Oxygen will be delivered to the affected wounded area through a type of hydrogel. The consumer will apply this gel which will be delivering oxygen for a certain amount of time period. Oxygen will need to be delivered at an ideal oxygen level over the duration of the application (5% to 10%.)

### **3.2.3 Consumers**

The wants of the consumers and what they are looking for in a product is also an important objective. This is because their opinion matters, since they are the potential candidates for this drug. If the consumers do not like the idea of having oxygen being administered in a topical product or applying a foreign product to their body can be hard to convince them to buy and try the product. The product will not need to be odorless, due to the fact that if the product creates a certain type of odor on their body, they will restrain themselves from using the product. As stated before, the product will need to be safe for consumers of all types of skin. If it is safe on sensitive skin, it will limit the irritation or rashes that it can create.

### **3.2.4 Quality Control**

The quality of the final product is important because it needs to ensure the same consistent quality and results from batch to batch. The quality of the finished product is not only based on the function or performance, but it is also based on the appearance and the quality of the ingredients that make up the product (Ueda, 2010). A visual examination that is performed on the product should identify if there are any changes in color, viscosity, or any separation of the ingredients (Ueda, 2010).

### **3.3 Functions**

The functions of the design serve to meet the design requirements. The list below (list #2) shows the four major functions that the product must comply with. The wound dressing should be self-sufficient, requiring no external attachments or professional help in applying, a barrier for the product, as well as how flexible it will be with the barrier.

1. Produce Oxygen
2. Ease of Use
3. Barrier
4. Flexibility

List #2: Main topics for functions

#### **3.3.1 Produce Oxygen**

The primary function for the purpose of this design is the production of oxygen. Oxygen is a major component for this product since oxygen is a key factor in the wound healing time. Oxygen is meant to decrease the average time it takes for a wound to heal. Oxygen is a requirement for various processes in the healing of wounds including collagen deposition, epithelization, fibroplasia, and angiogenesis (Castilla, Liu, & Velazquez, 2012). A few other functions of oxygen are also to kill bacteria and reduce infections (Castilla, Liu, & Velazquez, 2012). Oxygen stimulates the creation of new blood vessels and aids growth factors to form new skin (Castilla, Liu, & Velazquez, 2012). The design will incorporate a method of maintaining a desired level of oxygen in the wound area.

### **3.3.2 Ease of Use**

Since the consumers will be using this product it needs to be the simplest form of application. The product will be easy to use at the convenience of their house. This product will not need any medical intervention or seek any medic to apply this product for them. It should be designed so that the user could apply the product and cover the wound without additional assistance from machines or professional help. This product will also not require any knowledge of how to use any devices or technological materials.

### **3.3.3 Barrier**

Another function that is crucial to promoting a proper wound healing process is preventing infections. The design should function as a barrier on application, separating the wound area from the environmental surroundings so that its primary function of oxygen production and delivery to the wound may be uninterrupted.

### **3.3.4 Flexibility**

Its flexibility should aim to adapt to the user's skin and adhere properly, an additional consideration to keep in mind for the barrier.

## **3.4 Standards**

Standard for a product are ideas which are designated to be used as guideline or rules. Standards are chosen by what the new product will be composed of. Since this product will be a topical product, the standards that must be followed are standards for the skin, as well as pharmaceutical standards. Some of the few standards that must be followed are listed below (list #3) It should also

be able to compete with the standards set by current products on the market such as durability, ease of use, and water resistance. Some of the current products in the market which are like the product being spoken of are hydrogels and Hyperbaric Oxygen Therapy (HBOT). The most similar product is Hyperbaric Oxygen Therapy since it uses the major component that is being used, oxygen.

1. Dermal Safety Testing
2. Hydrogels and Hyperbaric Oxygen Therapy
3. Labeling and Packaging
4. Quality
5. Storage

List #3: Main topics for functions

### **3.4.1 Dermal Safety Testing**

Dermal safety testing must be done in order to assess the risk of the investigational drug. The tests will assess the tolerance of the drug under exaggerated conditions. Materials that are to be in contact with the skin should not cause any damage to the area it will be applied to or cause any irritation. The topical device must be biocompatible to skin, as well as the wound tissues it will be applied to. According to the ISO10993-1, “Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process,” it states that the product must be also tested in the final condition that it will be used (FDA, 2016). There are tests that need to be completed in order to determine the safeness of the product, as well as if it is biocompatible with humans. The first test that may be conducted is cytotoxicity, which is the extent to which the

material will possess a specific destructive action of the cells being used. When conducting the cytotoxicity test there are three characteristics used to determine the method that will be used: extract, direct contact, indirect contact. One type of cell that may be used are mammalian cells, and they can be incubated for a certain time period. After the incubation period is complete the cytotoxic effect is evaluated based on the table below (table #1).

*Table 1 Qualitative Morphological Grading of Cytotoxicity of Extracts*

Grade	Reactivity	Conditions of all Cultures
0	None	Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth
1	Slight	Not more than 20 % of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50 % of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50 % growth inhibition observable.
3	Moderate	Not more than 70 % of the cell layers contain rounded cells or are lysed; cell layers not destroyed, but more than 50 % growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

ISO 10993-10 part 10: “Tests for irritation and skin sensitization,” states the procedures and the limitations for irritation on the skin. This will determine the probability that the product will cause irritation to humans when they are using it. The site chosen for the test exposure would be to use the most appropriate and sensitive for the indicated use. For the product being explained here, the site to use would be the skin. Other sites would include the eye or mucosal membrane. Once these

tests have been conducted, they are evaluated and rated on the scores being shown in table #2. Different mean scores derived from the table #2 has its own response category. A mean score of 0 to 0,4 will be considered negligible. A score from 0,5 to 1,9 it will be considered slight irritable. A mean score of 2 to 4,9 will be considered moderate. Finally, a score of 5 to 8 will be considered sever.

*Table 2 Scoring System for Skin Reaction*

Reaction	Score
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema Formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond exposure area)	4
Maximal possible score for irritation	8

ISO 10993-10 part 10: “Tests for irritation and skin sensitization,” states the procedures and the limitations for another type of testing is sensitization. The sensitization test will test the potential



of a material or product to cause a sensitizing effect or allergic reaction in a patient over an extended period of exposure. This test is used when the product will come in direct contact with the skin, and the duration of the tests will last for a period of 5 weeks. Once the five weeks, have passed the results are given a grading scale based on the notable appearance of the affected area. There are two tests associated with this type of standard: The Guinea Pig Maximization Test (GPMT) and the Closed Patch Test. GPMT. The maximization test is the most sensitive and will produce the most accurate results. Extracts using 0.9% saline and cottonseed oil are prepared from the product. The test is performed in a series of three stages extending over a period of 4 weeks. The closed patch test is used mostly for materials that will have direct skin contact. It is performed in a series of phases over a period of 5 weeks.

*Table 3 Magnusson and Kligman scale*

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and/or swelling	3

### **3.4.2 Hydrogels and Hyperbaric Oxygen Therapy**

Current treatments available that are related to this project are hydrogels and hyperbaric oxygen therapy (HBOT). HBOT is a chamber in which the air pressure is increased higher than the normal air pressure. Once the air pressure is increased, the lungs will be able to gather more oxygen than what they are capable of breathing (Mayo Clinic, 2018). In 21 CFR 878.4022 it states a few regulations that the hydrogels must follow. These regulations are that hydrogel wound dressing

may not contain any additional drugs: antimicrobial agents, growth factors, or material derived from animals. The hydrogel must be composed of hydrophilic polymers or other material in combination with water. The range that the composition of the hydrogel must be is based on the percentage of water, which must be of at least 50 percent (FDA, 1999). There are a few functions that this hydrogel must be able to perform: absorb wound discharge, control bleeding, control fluid loss, and to protect against anything rubbing against it. For the HBOT there are many more different requirements that must be followed since this involves a step-by-step procedure to be performed. One of the major requirements is that medical directors must meet all the qualifications required. The Food and Drug Administration (FDA) has cleared hyperbaric chambers for certain medical uses, such as treating decompression sickness suffered by divers. During the procedure the minimum number of team size is of two people: the hyperbaric physician, the operator.

### **3.4.3 Labeling and Packaging**

The labeling regulations are different for over the counter drugs compared to prescription drugs. For labeling and packaging there must be written up documents that depict the steps that need to be followed to ensure the correct labels and packaging materials. They each have their own separate regulations and requirements that need to be followed. In the document 21CFR211 section 211.125 Labeling issuance, it depicts the strict control there is on the labels being issues for the use in the drug products. The batch that is set to be labeled should be carefully examined and identify if it conforms to the labeling specified in the master production records. Once there is a discrepancy found the entire batch will need to be investigated according to 211.192. Another standard that is stated is that once there is an excess amount of labeling or control numbers must

be destroyed and not put away. If a label has been returned, they should be classified and stored elsewhere. This will avoid any mix-up with the new labeling being used (FDA, 1995).

#### **3.4.4 Quality**

Thus, product quality may be assessed in terms of quality of the physical chemistry make-up of the finished product and the physical performance of the finished product. Product quality testing separated into two separate groups of tests: product quality tests and product performance tests. Three Test Categories for Semisolids: Universal Tests, Specific Tests (as appropriate/applicable) and Specific Tests for Topical Semisolid Products

According to 21 CFR 320.1 it states that there are two requirements that need to be followed which are bioavailability and bioequivalence. Bioavailability is the rate at which the active ingredient is absorbed from the product and becomes available to the site of action. For the products which are not intended to be absorbed by the bloodstream also need their bioavailability to be evaluated. This can be measured by the rate at which the active ingredient is available at the site. The bioequivalence requirement is mainly by FDA which involves two testing methods (in-vivo and in-vitro testing) of the product which must satisfy the condition that it is intended for the patients. Another requirement is the same drug product formulation. This means that the product being provided must have a constant composition from the definition formula submitted for approval. There may be some differences, but it must be similar enough to the bioequivalence provided.

### **3.4.5 Storage**

There are also different standards, requirements, and testing that need to be followed to assess the stability of the product. The stability will include determining the expiration date as well as what the desired storage conditions are. In 21CFR211: “Current Good Manufacturing Practice for Finished Pharmaceuticals” it states that there should be a written document discussing the standards and the conditions that need to be followed. The written document shall be followed and shall include the results of the testing that has been conducted. These shall include but are not limited to the sample size used, the storage conditions determined, and the test methods performed (FDA, 2016). Some standards of the testing that must be followed is that the drug product will be tested in the same closed system that the drug will be used in the market. The testing will be conducted by reading and using it as directed in the label. Enough batches for the drug product must be tested to determine the appropriate expiration date and a record of such data shall be maintained (FDA, 2016). In order to determine the expiration date there must be different drug product testing conducted at different intervals.

### **3.5 Constraints**

There are a few constraints that limit the objective of the design, as well as that the team must consider through this design process. The overall meaning of a constraint is something that will impose a restriction or prevent this product from being fabricated. These constraints are listed below:

1. Size of Application
2. Cost
3. Safety
4. Comparisons to the Market

List #4: Main topics for constraints

### **3.5.1 Size of Application**

The size of the application will be a constraint due to the fact that it will need to be able to cover an area of at least 2cm by 2 cm. The safety of the consumer is also a constraint for the project in which we need to take every risk that can occur into consideration. The product must be biocompatible with the skin, in which there must not be any cytotoxic materials or compound.

### **3.5.2 Cost**

Cost and financial availability is a constraint due to the fact that the product will need to go through various amount of testing. There is limited amount of materials being provided and limited amount of money provided. The amount of spending amount available is \$500 for the entire team. There are machines or devices that would help this project in obtaining better results as well as obtaining more information that would help justify that this product works. Another constraint will be that if there are expensive materials or the cost of production is high, this will not create a cost-efficient price for the consumers.

### **3.5.3 Safety**

Safety is a concern for this product because of the ingredients that are going to be used. Most of the safety testing will not be able to be performed or accessed, due to the time-constraints of the project.

### **3.5.4 Comparisons to the Market**

Furthermore, there isn't any product or device on the market currently that achieves our objectives. The product needs to be safe for the consumers to use, deliver oxygen, provide a range of potential consumers, and ensure quality control. As such, we do not have any similar products to refer to for analyzing the standards and specifications given. The standards and specifications we are trying to achieve are determined by ourselves in the design process within limitations given the time and resources we have. Our team still aims to analyze many wound dressing and application products that are relevant to our design objectives. Understanding the constraints of similar products will allow us to better determine what is feasible and what should be prioritized in the design like cost of production and manufacturing capabilities. There is not enough demand in the market to match the supply of your product. There is not enough supply of your product to match the demand in the market.

## **3.6 Specifications**

The specifications listed below were derived from the function that the design will need to satisfy. A specification is a detailed description of the design and materials that will be needed to make the product function.

1. Storage
2. Resistance to elements
3. Oxygen Production

List #5: Main topics for specifications

### **3.6.1 Storage**

As such, the product should also have a long shelf life and easy storage for the convenience of the consumer. The shelf-life for the topical product being used will be of at least 18 months in order to potentially compete in the market versus similar products. Some of the similar products have been discussed before which are hydrogels being used for wounded areas. The shelf-life has been determined due to the fact that the shelf-life of a hydrogel has been determined to be 18 months under ambient conditions (Bates & Magda, 2014).

### **3.6.2 Resistance to Elements**

The amount of sunlight the product will need to be exposed to is at most 45% of the sunlight. Our team plans to do a multitude of stress tests to determine what material is best for the composition of the design, considering how much should the product be able to handle. We will be testing for mechanical strength, shear stress, strain, flexibility, etc. The material should be able to contain the necessary substances like a gel for holding the chloroplasts while safely adhering to the user's skin. Tensile strength and flat plate water trials are the types of tests we plan to conduct for material choices and measure the specifications needed for the design.

### 3.6.3 Oxygen Production

One measurement to take note of is how much oxygen can be produced over a certain volume or surface area. One of our objectives is to ensure that the product is easy to apply for the consumer without the need for professional help.

### 3.7 Revised Client Statement

After adjusting the objectives based on the client's needs as well as through research conducted from current literature, the client statement was able to be revised. The client statement now reflects the overall goal of the project, as well as the main objectives that this product needs to produce. The revised client statement is:

“Design a new topical treatment, which will produce oxygen for 24 hours to the affected wounded area. This treatment will be safe for the patient to apply at the convenience of their home. The product will be able to be stored for at least six months. The topical treatment product will be consistent with and ensure a broad range of potential consumers.”

### 3.8 Management Approach

In identifying different stakeholders in our project, clarifying objectives, and considering our constraints, the preliminary design will be developed for a prototype and testing. Even though no marketed products offer the capabilities sought by our client, comparison charts and analysis can still be conducted to determine the competition and global standards for wound dressings and



similar products. The characteristics of our final design will determine if we follow the regulations established for medical devices or pharmaceutical methods.

In the prototyping phase, the logistics will be considered for making material choices for testing and implementing with the inclusion of chloroplasts for oxygen production. This phase will be crucial for evaluating our limitations and any issues with the design from a conceptual standpoint and a development standpoint. Following up on the testing phase is meeting the regulatory standards to potentially put this product out on the market. Financial aspects such as cost-effectiveness in production and monitoring in quality control are future considerations.

Below is an outline of a Gantt Chart (first half of the project year) subjected to change as we work through the phases of the project. Workarounds will be expected depending on any unexpected setbacks as we aim to narrow down our scope to focus on the primary objectives of the project.

The time available for the project is a major constraint in that we need to have everything completed and ready to submit by April. This gives us about 6 months to complete the production, the testing and if something were to fail during the testing, we would need to perform further alterations. The time available for the product to be produced is also limited since we have until April to submit our results, this means that we need at most two months prior to this for testing. Taking this into consideration, the product will need to be produced and ready for testing by January. The team also shares a budget of 500 USD during the project design. This budget entails for any traveling to conduct research or any potential interviews, as well as using it to construct and test the prototype.

## **Chapter 4 Design Process**

Here it is discussed the process of determining the final design process. The objectives, goals and functions were all weighed out and determine the importance of each one. The basic concept map is depicted here as well as the detailed concept map. The three different experiments are also discussed.

### **4. 1 Needs Analysis**

After ranking the design objectives and developing the client statement, the design team began to organize a list of functions for the scaffold that would satisfy the desired objectives. The team began by drawing from the literature review to brainstorm an initial list of functions and design specifications. Brainstorming with the client was used to inspire means and approaches to the conception of the scaffold. Afterwards, a preliminary function and means list was generated and eventually pruned to better fit the client objectives.

#### **4.1.1 Pairwise Comparison**

The team ranked the objectives described in chapter 3 using a pairwise comparison chart, as shown below in Table 4. A pairwise comparison chart will determine the importance of the design objectives by listing the objectives across the first row and down the first column of a table. Then the objective in the column is compared against the objective in the top row. When there is a number 1 in either box this means that the objective in the column is more important than the objective listed in the row. When there is a number zero this will be the opposite, the objective in the column will be less important than the objective in the row. Once the boxes are all filled up, a

total number is achieved from adding across the table. This will indicate the weight of each objective. For table #4, the most important objective was found to the least important: safety, delivery of oxygen, quality control and consumers. This process was repeated for the goals, as well, which can be seen in table #5. The order of importance for the goals was ranging from production of oxygen, ease of use, barrier and finally the least important is flexibility.

Table 4 Objectives

	Safety	Delivery of Oxygen	Consumers	Quality control	Total
Safety	x	1	1	1	3
Delivery of Oxygen	0	x	1	1	2
Consumers	0	0	x	0	0
Quality control	0	0	1	x	1

Table 5 Goals

	Produce Oxygen	Ease of use	Barrier	Flexibility	Total
Produce Oxygen	x	1	1	1	3
Ease of use	0	x	1	1	2
Barrier	0	0	x	1	1
Flexibility	0	0	0	x	0

## 4.1.2 Ranking the Requirements

Table 6 Requirements

Requirements	Team	Client	Total
Safe for the patient to apply to their skin	5		
It will meet the client's needs and or wants	4		
There will be oxygen flow	5		
It will ensure a range of potential consumers	2		
The quality control will be kept the same	4		
It will produce oxygen	5		
It will be able to be applied by the consumer (no medical intervention)	3		
It will need a barrier or covering after each application	3		
The covering or barrier will be flexible	1		
The product will be cost-effective	2		
Storage for an extended period (6 months)	1		

5 (Must Have)

The final product must be able to produce oxygen for a set amount of time. As well as have constant oxygen flow on the affected wound area. This produce must be safe for any patient or consumer to use and will not create any reaction to the product.

#### 4 (Important)

The second goal is to meet the client's needs or wants without affecting the consumers well-being.

The second goal is for the batch created to have a consistent amount of oxygen production. There will not be any major fluctuations of oxygen being produced.

#### 3 (Good to Have)

By creating this product, it will be able to be applied by the consumer himself. This will not need any medical intervention, or any external technology or devices used. The hydrogel that is selected for use within the system

could be used with an external covering to avoid any further contamination of the area.

#### 2 (Not Needed, but Would Be Nice to Have)

An additional goal is to provide a low cost and cost-efficient for low income class consumers. This will also ensure a broader range of consumers. Ensuring a broader range of consumer can increase the sale of the product and the viability of it.

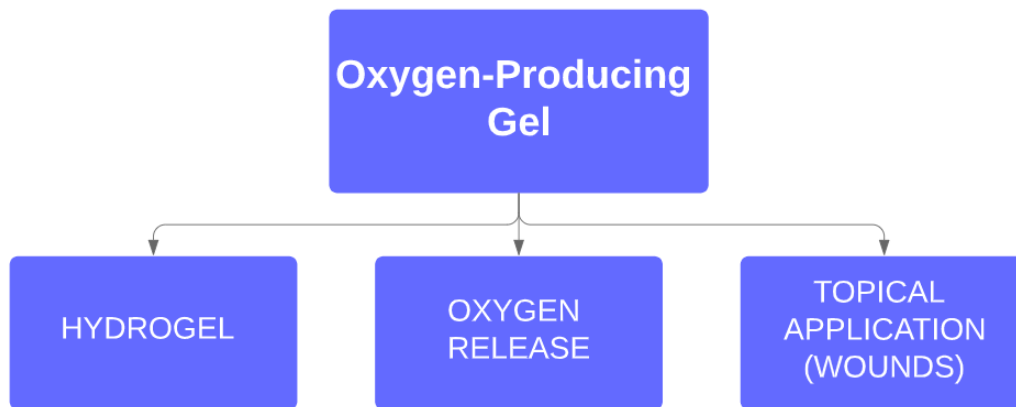
#### 1 (Not Necessary)

The last goal is that if a covering is being used it will not produce any discomfort for the consumer.

The barrier will be un-noticeable as well as easy to apply on top of the product. The storage time for the product would be great if it can be stored for at least six-month in its container without its function degrading.

## 4.2 Conceptual Designs

After determining the priority of the objectives, design constraints, goals, requirements and functions, a conceptual design was brainstormed. This basic conceptual design is shown in figure 1, which begins of with the main purpose of the product divided into three subsections. There were three main components listed for the product which are the hydrogel, oxygen, and the topical application.



*Figure 4-1 Basic Concept Map*

Even though the basic concept map in the figure above shows the fundamentals of the product, a more in-depth concept map was created as well, shown below in figure 2. This concept map is beginning with the main purpose of the product divided into the three main subsection: topical application, topical products, and oxygen producing. These three subsections will then be divided further into their own different sections which will help understand the product more in-depth. For the topical product, it was divided into the types of products that may be used. For the oxygen producing section it was divide into the light source needed as well as the mechanisms to measure the release of oxygen.

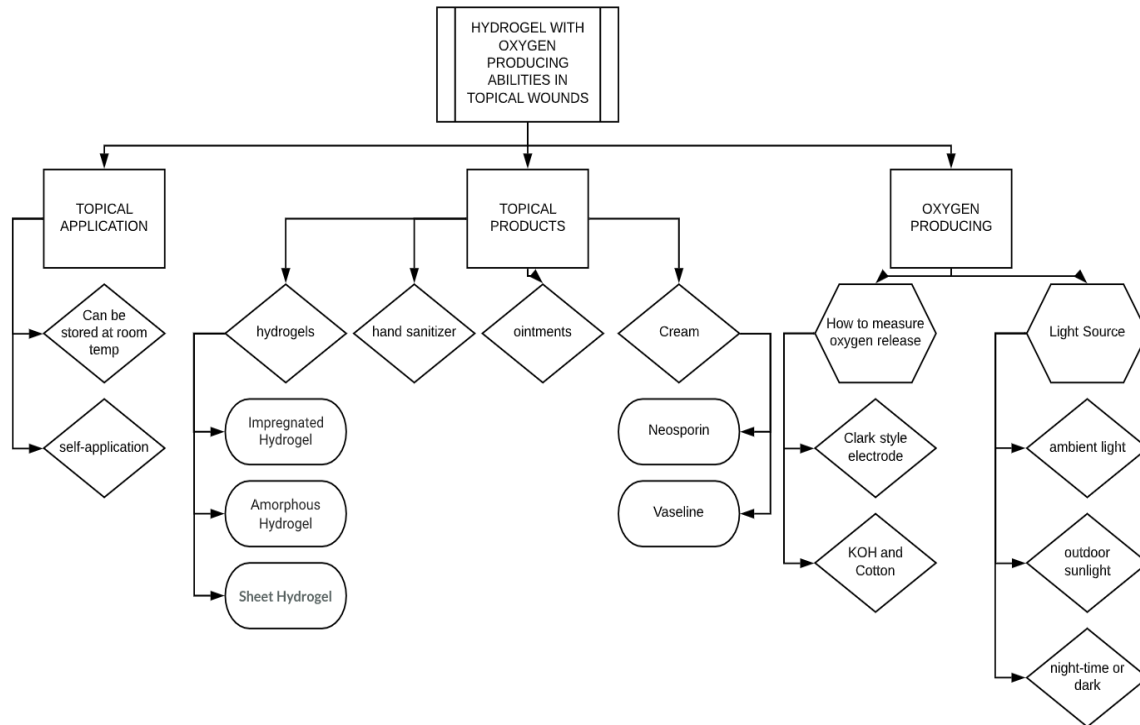


Figure 4-2 Detailed Concept Map

### 4.3 Alternative Designs:

At this point of the design process it is known the preferred design that would be best for this product. Therefore, we considered four alternative designs that would represent and follow the guidelines of our project. These alternative designs will satisfy the goals, objectives, and requirements that were set by the client statement. These designs will be used as backup design just in case the preferred design fails at some point or it is not producing the desired results. The designs will also comply with the concept map and can be used as future design and improvements for the product

#### **4.3.1 Vaseline infused gel**

This was the initial design chosen to use as the mixing agent with the chloroplast solution. The Vaseline was going to be used as its solid form, but it was also heated in order to dissolve it to properly mix it with the solution.

#### **4.3.2 Hand sanitizer gel**

Hand sanitizer was the second option to use as the mixing agent with the chloroplast solution. The hand sanitizer was not manipulated but used as it was from the bottle. This may not properly work due to the hand sanitizer having alcohol. Alcohol will kill the chloroplast cells and not make them viable in the solution.

#### **4.3.3 Glycerol Hydrogel**

Glycerol is the third back-up just in case the other two substances have not properly worked. Glycerol is an ingredient that is used in burn ointments. People are familiar with using this on their burn wounds as well as it does not contain any ingredient harmful to the chloroplasts.

#### **4.3.4 Band-aid infused with a layer of gel**

A band-aid infused chloroplast solution would have been great in creating. Due to limited time and materials this was not possible. The band-aid would have to be manipulated in order to allow any source of light to enter for the chloroplasts. The band-aids also have size limits while creams, ointments, and gels do not. They can be applied to any place and any size of area.



#### 4.4. Final Design Selection:

Table 7 Comparing Alternative Designs

	Time	Availability	Compatibility	Total
Vaseline	4	4	1	9
Hand-Sanitizer	5	5	3	13
Glycerol	5	5	5	15
Band-aid	3	3	5	11

As you can see in the table above, when comparing all the alternative designs there was only one design which stood out the most. Even though that design stood out, there were tests performed for Vaseline and Hand-Sanitizer in order to completely rule them out. Upon different types of testing Vaseline was the first to be ruled out. This was most due to the fact that it would not properly mix with the chloroplast solution. The second to be ruled out was hand sanitizer, this was due to the alcohol content that it has. Band-aid was also ruled out at the initial brainstorming because of the many changes that may have to be done to the band aid itself. Another change was how the gel was going to be incorporated in the band-aid without it seeping out and changing the integrity of the adhesive.

## Chapter 5 Design Verification

In this section the process of performing the experiments are explained. It is also explained how the final design was obtained and why the desired measurements were used for each product used.

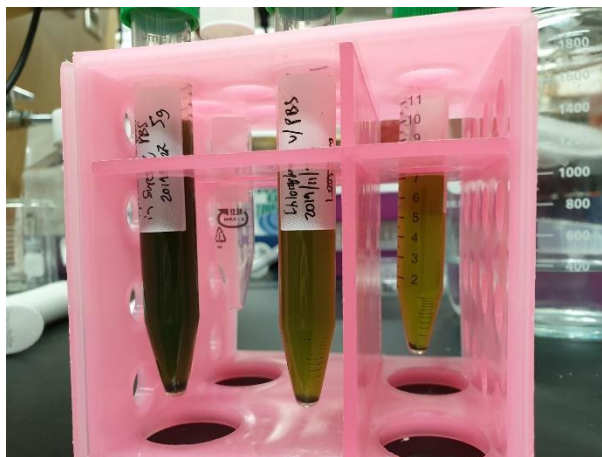
### 5.1 Initial Preparing

In order to begin with the preparation there was a need to verify the amount of each material needed. The batch for the final design was created by mixing five grams of spinach leaves with a solution that contained 0.5 M Sucrose x 1 Phosphate-Buffer Saline (PBS), filtering it and spinning the tube in the centrifuge. This isolated the chloroplasts and created a final batch containing 25 ml of solution.



*Figure 5-1 Initial Preparation Materials*

The batch for the final design was created by mixing five grams of spinach leaves with Phosphate-Buffer Saline (PBS), filtering it and spinning the tube in the centrifuge. This was spun at 2,000 rpm for 10 minutes. This isolated the chloroplasts and created a final batch containing 25 ml of final solution.



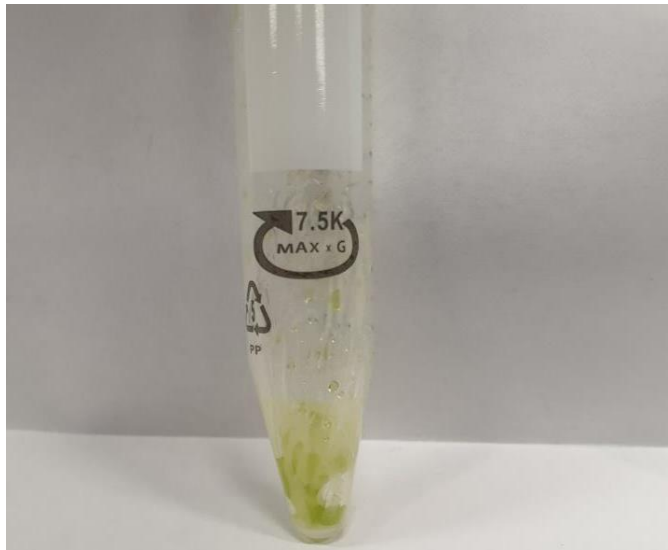
*Figure 5-2 Chloroplast Solution*

## **5.2 Choosing the Material**

In order to determine which product is compatible with the chloroplast solution there was one experiment performed. The hill reaction was performed to determine whether the product and the chloroplast solution are compatible with each other

### **5.2.1 Vaseline**

The initial batch was going to be tested with Vaseline. Vaseline is also known as petroleum jelly, and it is a hydrophobic molecule. Since it is a hydrophobic molecule it will repel the water. As shown in the figure below the Vaseline and chloroplast solution does not mix well together.



*Figure 5-3 Chloroplast Solution with Vaseline*

Due to this we decided to heat the Vaseline to determine whether in a more liquified state it will accept water. The Vaseline was heated in warm water, but when it was combined with water it did not mix well either, the results are shown below.



*Figure 5-4 Chloroplast Solution with Heated Vaseline*

### 5.2.2 Hand Sanitizer

Once Vaseline was not a good compatible with the chloroplast solution, the team moved on to using hand sanitizer. Hand sanitizer was compatible with water in which they bonded perfectly together. The alcohol breaks down the chlorophyll, taking the green color out of the leaf. After a day or two the alcohol will begin to kill the chloroplast.



*Figure 5-5 Chloroplasts with Hand Sanitizer*

### 5.2.3 Glycerol

The next option after trying to use Vaseline and hand sanitizer was incorporating glycerol. Glycerol is one of the main ingredients used in wound ointments that are for burn victims. Glycerol is a colorless, odorless, and very viscous liquid that is contained in a clear bottle. Glycerol is a non-toxic substance which is not harmful to the human skin. Glycerol has antimicrobial, antiviral properties and is widely used in wound and burn treatments.

## 5.3 Testing

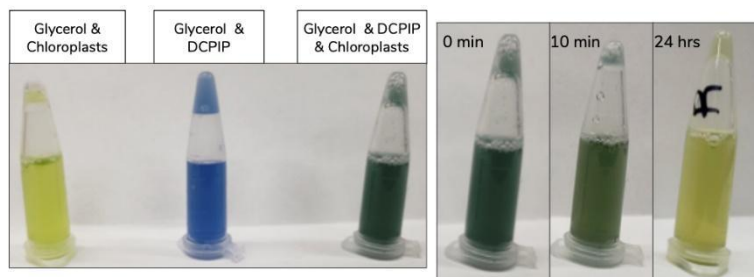
There were two types of testing done for this final product in which it helped us better observe whether the chloroplasts were still producing oxygen after a certain amount of time. These testing were to determine whether our chosen material, glycerol, will be compatible with the chloroplast solution.

### 5.3.1 Hill Reaction



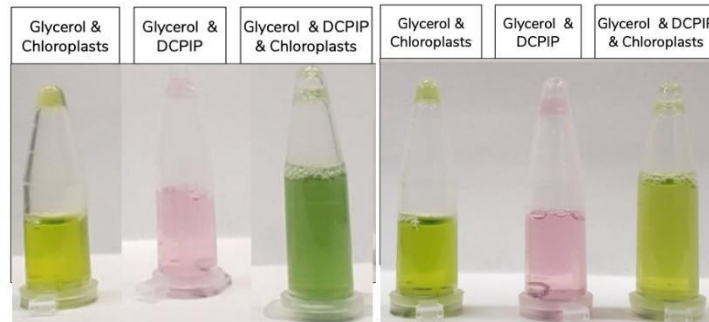
*Figure 5-6 Hill Reaction with Hand Sanitizer*

The first test was using the hill-reaction without using any light source, instead we just used the light which was around us. Without using any light source did produce oxygen, but it took longer for the hill-reaction to occur.



*Figure 5-7 Hill Reaction Under Ambient Light*

The following test was using the same amount of chloroplast solution while using a lamp as a light source. The changes are shown below in figure 6 which it took a less time for the hill-reaction to take place. The left image depicts the glycerol and chloroplasts at zero minutes while the right picture depicts it after 7 minutes of testing.



*Figure 5-8 Hill Reaction Under a Lamp: Left Initial at 0 minutes, Right After 7 minutes*

## **Chapter 6 Final Design and Validation**

The team analyzed the composition of the final design and determined what the steps of producing this product was. The team also analyzed the impact of this product on the environment as well as how it would change the countries. The team considered how the new hydrogel producing oxygen would perform on the economy, environment, society, politics, ethics, health and safety issues, manufacturability and sustainability. This product will have both positive and negative impacts on these issues.

### **6.1 Final Design**

After performing these different types of experiments. There was one weight of the spinach leaves which was enough to function properly with the chloroplast solution. These steps and materials are explained in the following subsections.

#### **6.1.1 Isolating Chloroplasts**

The spinach leaves will be weighed out to measure around five grams using the scale. Once the leaves have been weighed out, they will be crushed in the mortar with the pestle. This will be done until the leaves are fully crushed. Once the leaves have been fully crushed, there will be 25 ml of PBS added into the mortar. The leaves will be crushed some more. Here you will begin to notice the PBS will begin to turn a slight green color. Once this has been properly crushed and mixed it will be filtered using a coffee filter into a centrifuge tube. The coffee filter will be inserted into a funnel to make the process easier in pouring. The solution will fill up the centrifuge tube and it will be spun at 2000 rpm for 10 minutes. After 10 minutes you will have noticed that there was



still some dead cell wall and other foreign substances that passed through the coffee filter. You will aspirate the solution into a new centrifuge tube without aspirating any substance that has settled on the bottom of the tube. You will take 7 microliters of solution and use a hemocytometer to analyze whether there are chloroplasts present in the solution.

### **6.1.2 Preparing the Solution**

Once it was confirmed that there were still viable chloroplasts present in the solution now the solution will be prepared. This is the final part of preparing the hydrogel. The solution will be a one to one ratio of glycerol and the chloroplast solution. Since grams and milliliters are proportional to each other in that 0.5 grams will equal 0.5 milliliters. So, when weighing out the glycerol the boats were used, and the balance was zeroed every time a new measurement with a new boat was used. The glycerol was added slowly into the weigh boats to enable proper measuring. The chloroplast solution was measured by using the micropipette tools.

## **6.2 Impact Analysis**

There are many different factors which come in play when creating a new product. These steps both have positive and negative impact on the product, economy, society, and environment. There are also many ethical problems which come into play when there are tests performed with these new products.

### **6.2.1 Economics**

There are varying materials for different types of wounds. For example, for burns there are hydrogels and ointments that both are to help relieve the pain and heal. As you can see in figure

10, in 2019 the United States sold about 42.1 million first aid tape/ bandages (Shahbandeh, 2019). Since health care costs have been increasing throughout the years, in 2009 about 25 billion dollars was spent on treating chronic wounds (Sen et al., 2009). The cost of a standard dressing would cost around 24 dollars, when the dressing is changed every other day. So, for a six-month period of using a dressing it would cost about 3,500 dollars. This cost is without the use of any dressing or ointment. If there was a dressing used it would cost about 30 dollars a use, resulting in about 1,000 dollars in three months (Al-Gharibi, Sharstha, & Al-Faras, 2019).

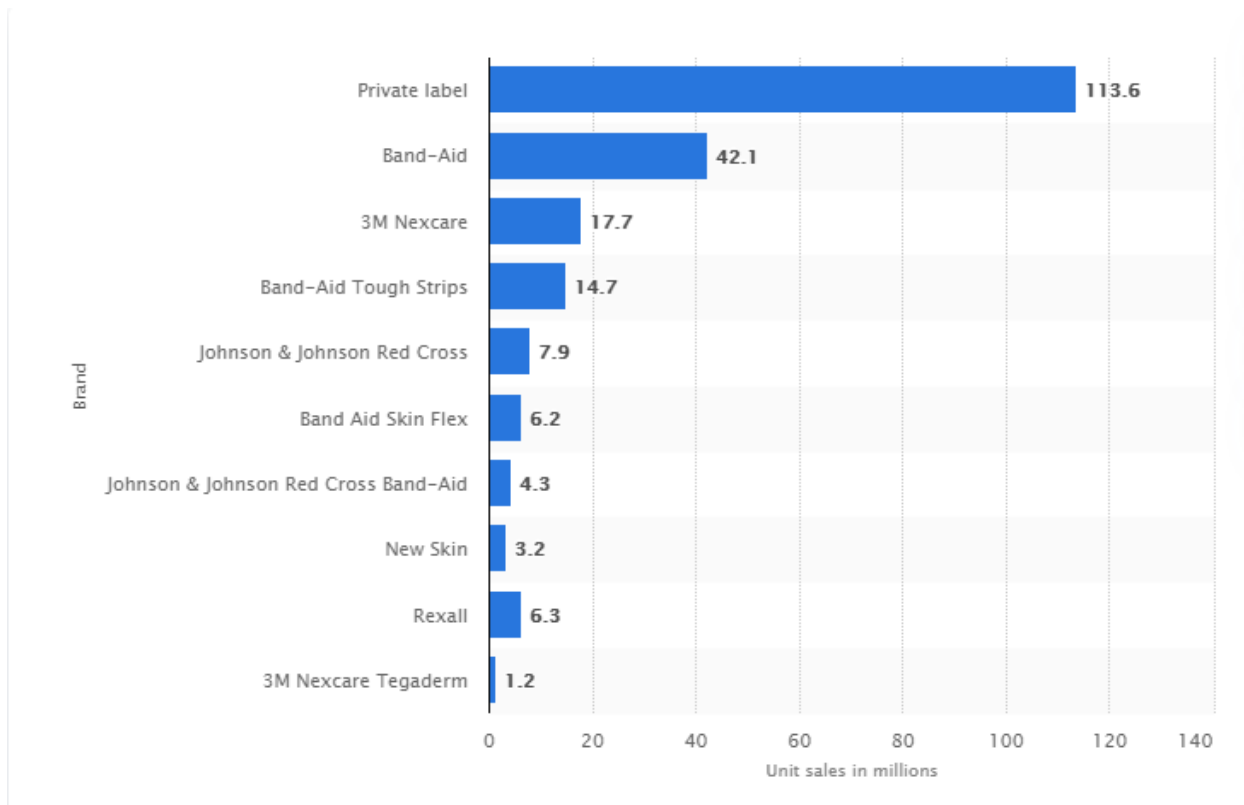


Figure 6-1 Unit sales of Brand Names (Shahbandeh, 2019)

## 6.2.2 Environmental Impact

There are many positive effects of having this naturally made hydrogel. As we all know planting trees have a great impact on the environment as do planting small plants. Once a spinach plant is

plants it will begin to improve the quality of the air as well as the humidity levels. Plants as well as trees also aid in noise pollution, in that they absorb the noise around them. When there is a significant amount of pollutants plants will absorb these and convert it to food. Plants absorb the pollutants from the air through their stomata. Once the pollutant is transported to the root it will biodegrade the pollutants into structure which they will use as food (Benefits of Plants).

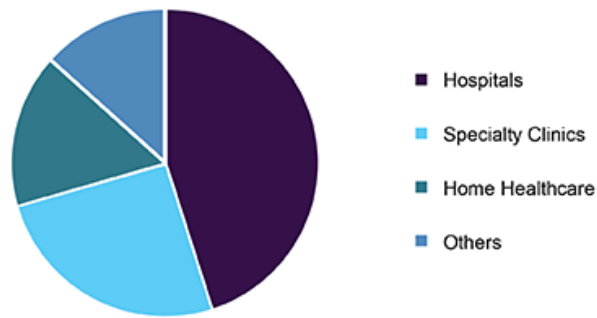
### **6.2.3 Societal Influence**

This new oxygen producing hydrogel will have a great impact on society. Currently in the market there are no hydrogels which produce oxygen. The only oxygen producing device in the market is the Hyperbaric Oxygen Chamber. There are many disadvantages with this device in that the patients must go into the medical office to get the treatment. The cost of these treatments would range from thousands of dollars and there would be many sessions in order for a wound to fully heal. In using a hydrogel, it will provide less scarring than using other ointments. This is especially important in cases of burn victims. Their trauma of being burnt and how they will look with all their scars will increase high depending on the severity of the wound.

### **6.2.4 Political Ramifications**

Since the United State and some other major countries are the leading countries in producing topical wound care it will benefit these countries the most. As you can see in figure 12 hospitals are the largest share which uses wound care products followed by clinics, home healthcare and finally others.

**Global wound care market share, by end use, 2018 (%)**



Source: [www.grandviewresearch.com](http://www.grandviewresearch.com)

*Figure 6-2 Global Wound Care Market Share (Wound Care Size, 2019)*

### **6.2.5 Ethical Concerns**

There are some ethical concerns involved with this new hydrogel that was created. The main concern that may be available is the amount of testing that needs to be done on animals. Improper animal care and the protocol that must be followed to test the product on the animal will concern many people. The protocol will need to be followed according to the Institutional Animal Care and Use Committees standards (IACUC). The staff who oversee performing the experiments must be familiar with the proper animal care as well as the specific ways to bring up any concern to the IACUC. The IACUC has a special board which oversees receiving the complaints and investigating them.

### **6.2.6 Health and Safety Issues**

There are many ways that a new topical wound care product can help patients with their health and safety issues. Some ways can include improving their self-esteem by calming them and reducing any negativity they may be holding in. This is important since stress has a major impact in wound

healing. When a patient is stressed or anxious their wound healing process will be impaired. Stress will impair cellular immunity, which compromises wound healing (Sen, 2019). Patients will not be as secure in applying a new topical product to their wound when they are aware that they contain chloroplasts in them.

### **6.2.7 Manufacturability**

Since our product has potential in healing patients with wounds at a faster pace there are some limitations in manufacturing. The chloroplasts will require it to be under a light source to activate it. When it is under ambient light the reaction process takes a longer time. Materials, equipment, and labor will be required for isolating the chloroplasts and combining the materials together.

### **6.2.8 Sustainability**

Since our product is almost completely naturally-made the only materials required to produce the hydrogels are spinach leaves, glycerol and PBS. When fabricating these gels, you will also need pipettes, plastic and glass tools, ceramic mortar and pestle. These materials do not operate on using renewable energy. In contrast to this, the production of this will require little to no energy as well as it can be stored in a refrigerator until it will be used. If the materials used were to be disposable and use renewable energy than this will be more sustainable to the environment as well as it will help the environment and not harm it.

## Chapter 7 Discussion

The goal of this project was to develop a method of utilizing the functions of chloroplasts to naturally produce oxygen and promote wound healing. Our results have shown that amongst all the preliminary design choices, glycerol was the best fit to meeting our design objectives and constraints. As mentioned beforehand, glycerol is colorless, odorless and most importantly, non-toxic. As it is not harmful to the human skin, this design choice met our criteria for developing a safe method to host the chloroplasts for oxygen delivery. Despite the lack of in-depth knowledge of the role of oxygen in the molecular and cellular level, studies have shown that oxygen does play a major role in the process of wound healing.

As our testing has shown that the chloroplasts were able to sustain their functions and continue to produce oxygen per the Hill Reaction, this design choice considers both the objectives of taking advantage of oxygen and the constraints of maintaining safety and ease of use. It was clear that our testing with using Vaseline to host our chloroplast suspension was unfit for our standards to the heterogeneous mixture it resulted in. This is due to the hydrophilic properties of petroleum jelly which meant it was insoluble with our experimental chloroplast solution. The choice of hand sanitizer was able to host the chloroplast suspension temporarily as the alcohol started to kill off the chloroplasts after 1-2 days. Hence, this choice also did not meet our design standards despite being able to mix homogeneously with the chloroplast suspension. It was important to note how the results came about with the hand sanitizer mix. Our team had expected the chloroplasts to not survive for more than 24 hours in the mixture. The reasoning behind using hand sanitizer in the first place was to test how the chloroplast suspension would mix with the sanitizer solution at room temperatures. The mixture turned out to be homogeneous as expected,

but the team did not expect the chloroplasts to survive as long as it did in testing (1-2 days) when taking note of the high alcohol concentration.

Upon choosing our final design with glycerol as the material of choice, our team has to reflect upon our objectives and constraints to understand to what extent the choice of glycerol meets our criteria. After reviewing those conditions, glycerol appeared to satisfy the criteria without any drastic consequences. The choice of glycerol was able to contain the chloroplast suspension in a homogeneous mixture, is non-toxic, and has the potential to be cost-effective with our design protocol.

In regard to comparing our experimental results with other literature, it seems that there doesn't exist in-depth studies about chloroplasts and oxygen production. We were unable to directly analyze how efficient the oxygen production in the chloroplasts were due to the lack of existing research. However, it is important to note that based on our observations regarding our trial results, we can definitively conclude that oxygen production was present per the Hill Reaction as the chloroplasts were able to conduct photosynthesis with and without a concentrated light source. This portrays evidence that the chloroplasts still continue to function even after being extracted and suspended in a different environment.

Over the course of conducting these trials for chloroplast activity, we had acknowledged the limitations of our experiment. As mentioned previously, there were no in-depth studies announcing the volume and efficiency of oxygen production in chloroplasts, even more so with chloroplasts extracted from spinach leaves in our case. Furthermore, we realized that the trials we conducted only tested for extended chloroplast activity for no longer than a week. One of our design objectives was to develop a product that has an extended shelf life of up to 6 months in order to compete with existing products in the market. Although our results have its shortcomings

of being unable to accurately determine how long the chloroplasts will survive long after production of the product for wound healing, we did acquire data in that chloroplasts can still function and produce the oxygen needed for the wound healing process. Our data successfully reports that chloroplasts will continue to conduct photosynthesis and portray the Hill Reaction post-extraction.



## **Chapter 8 Conclusion**

The team's final design selection was in favor of proceeding with utilizing glycerol as the main component in hosting a chloroplast suspension for oxygen production and delivery. This decision was due to glycerol satisfying our criteria without any major consequences within our scope. Our results yielded a positive outcome as it was determined that it is possible to maintain the chloroplasts' functionality outside its natural environment. Unfortunately, the team was unable to produce a fully working product, as our scope focused on designing a protocol of utilizing the chloroplasts for wound treatment. The following discussion presents recommendations for overcoming our project limitations as well as future methods for realizing the successful production of our novel chloroplast-based method for wound treatment. This chapter will also reflect on the actions and tasks left undone due to certain circumstances, in hopes of pointing out another approach to solving the problem or improving upon our current work.

### **8.1 Future Work**

For any type of future work or experiments there are some new modifications that can work with this product. Some future work that may be performed is by experimenting with different ratio of glycerol. For the three-experiment mention that were previously mentioned there was only a one-to-one ratio of glycerol to the chloroplast solution. For the future work more ratios can be used more tests as well can be performed in order to measure the about of oxygen that is being released from the chloroplasts. Other future works that can be done on this product is different testing on a cell culture as well as on animals. For the cell culture there can be an experiment where a wound is performed on the cells and the product will be added to determine its rate of healing.

## **8.2 Recommendations**

Through the various experiments previously mentioned, the team was able to determine which methods work best for the product. The modifications were made in order to improve the product's validity. The following is a recommendation that we advise in order to better the design of our product. This recommendation will help the product be safer and more viable for patients to use.

### **8.2.1 Disinfection and Sterilization**

When a product is sterilized it adds to the validity of the product as well as it makes it safer for patients to use. There are many ways to sterilize a product and maintain its full capability. In appendix B it is shown the difference between sterilization and disinfecting a product. Sterilization is using chemicals, heat, pressure, filtration, and irradiation. Disinfection uses detergent, alcohols, bleach, as well as heating and pasteurization. Disinfection is less sterile than when you sterilize a product. This is because disinfection kill bacteria another small living organism that produce disease. While sterilization is the destruction of all microorganisms. In the image below it shows the ranking of sterilization and disinfection. Here it is also seen that sterilization is ranked better than disinfection, but prion reprocessing is higher and better than sterilization.

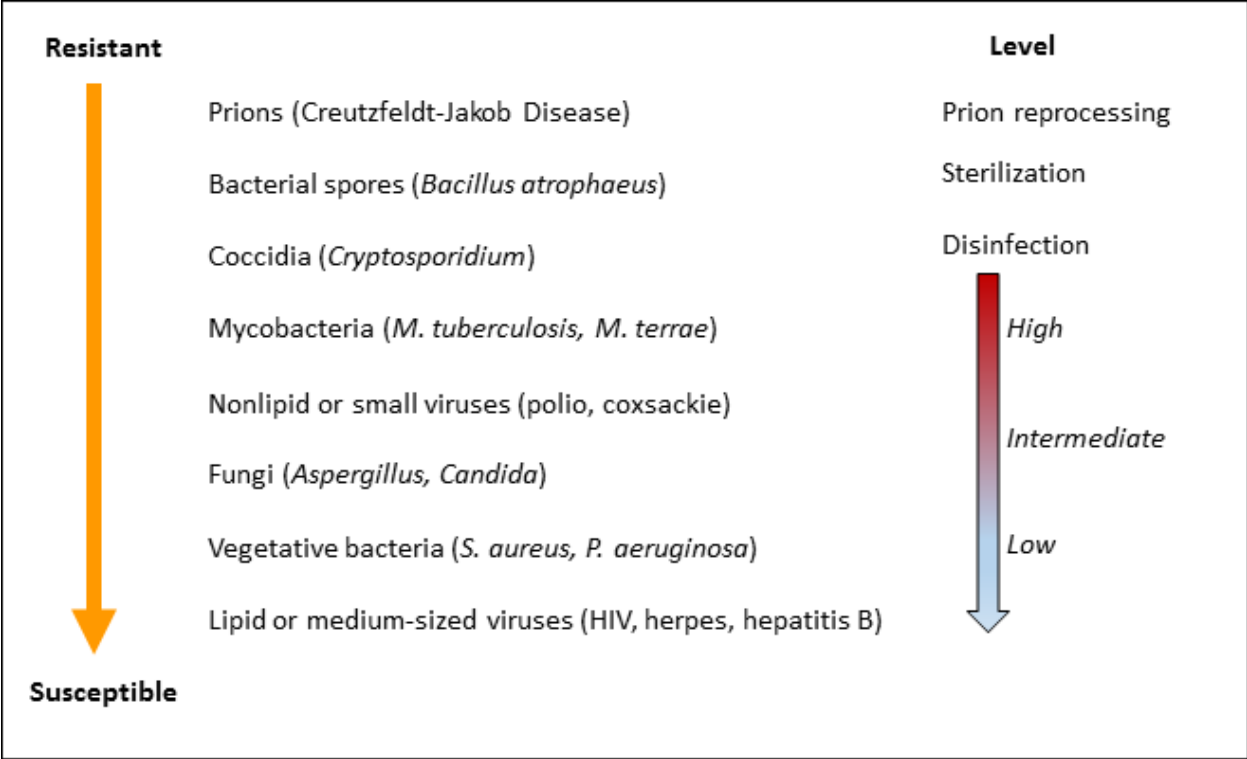


Figure 8-1 Ranking the Bacteria Killed (CDC, 2016)

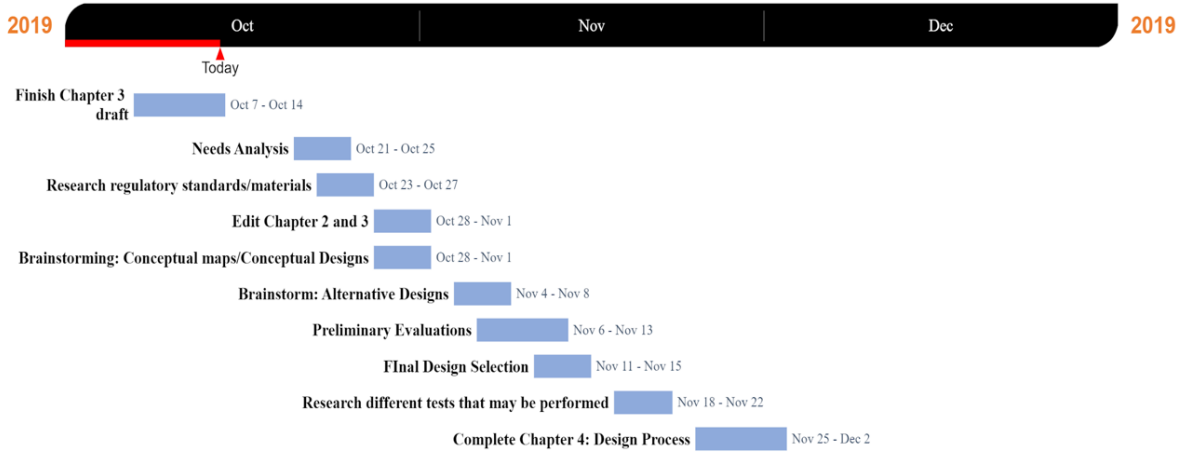
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# Appendices

## Appendix A: B-term Gantt Chart

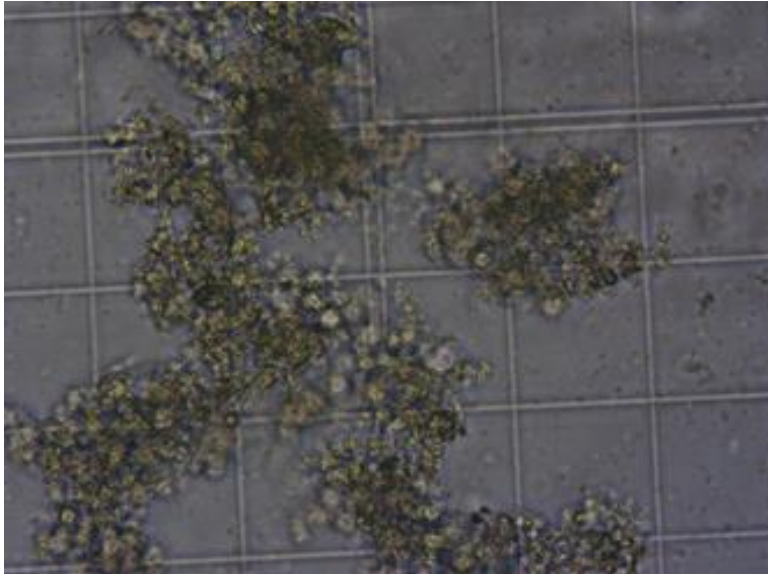


## Appendix B: Sterilization vs Disinfection

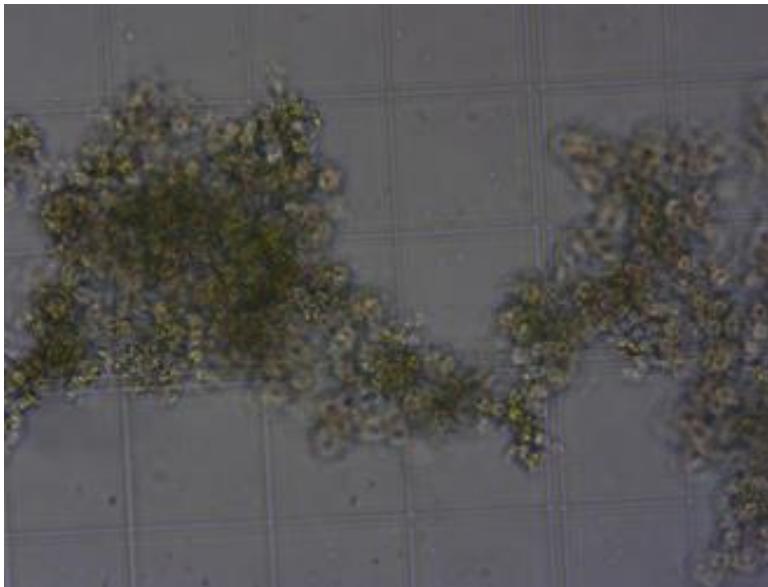
### STERILIZATION VERSUS DISINFECTION

STERILIZATION	DISINFECTION
Total destruction of all microorganisms (whether or not pathogenic) and their spores, usually through the use of drastic methods	Cleaning of something using chemicals that kill bacteria and other very small living things that cause disease
Uses more robust methods such as high heat or chemicals	Uses moderately effective methods
Uses chemicals, heat, high pressure, filtration, and irradiation	Uses detergents, hydrogen peroxide, alcohols, bleach, halogens like chlorine, phenolic disinfectants, heavy metals, heating and pasteurization
A method that gives extreme cleanliness	A method that gives an adequate cleanliness
Destroys both living organisms and their resistant structures	Destroys only the living organisms
Used in the decontamination of food, medicine and surgical instruments	Mostly used to decontaminate surfaces and air
	Visit <a href="http://www.PEDIAA.com">www.PEDIAA.com</a>

**Appendix C: Microscope Images Hand Sanitizer (40x)**

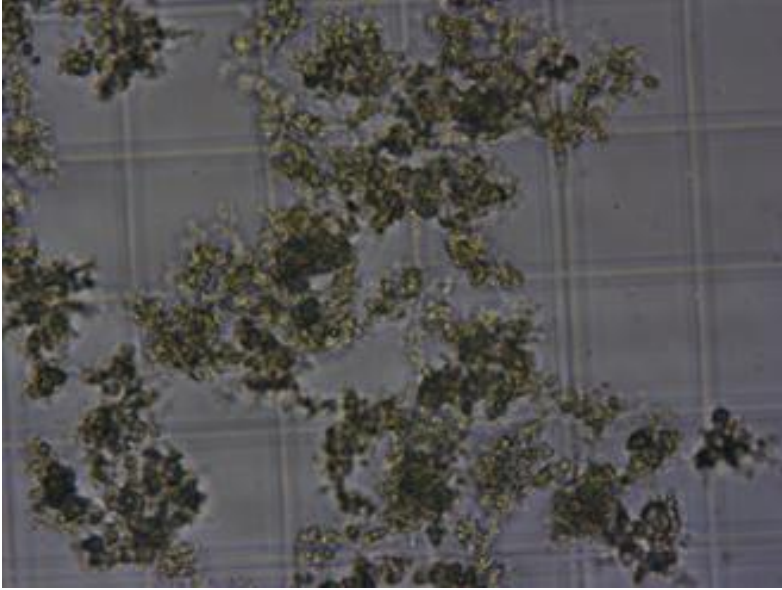


(Experiment 1)



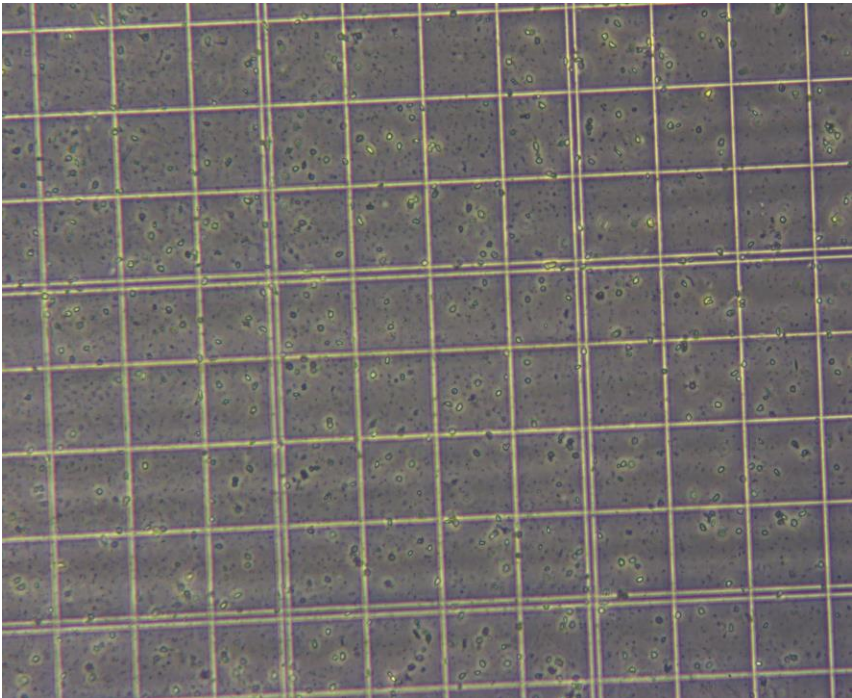
(Experiment 2)



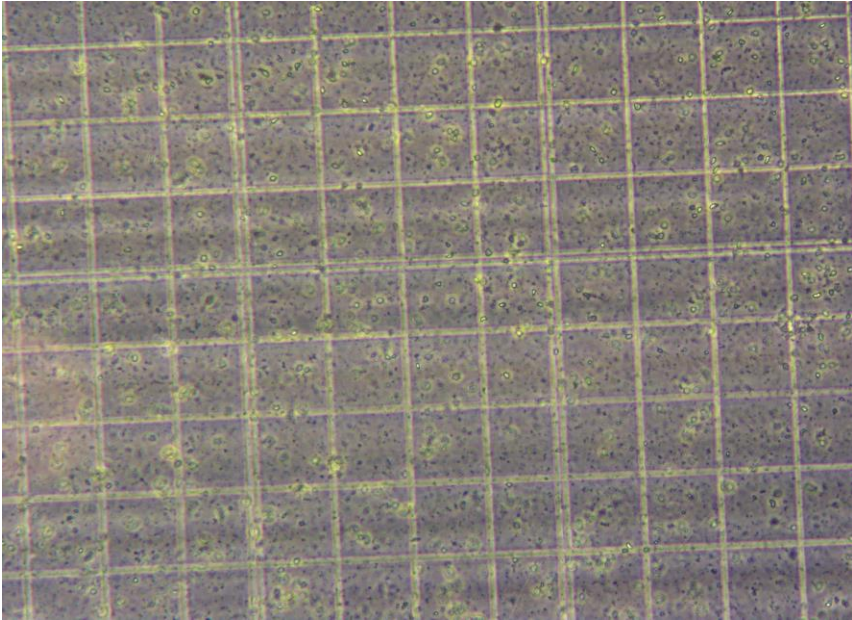


(Experiment 3)

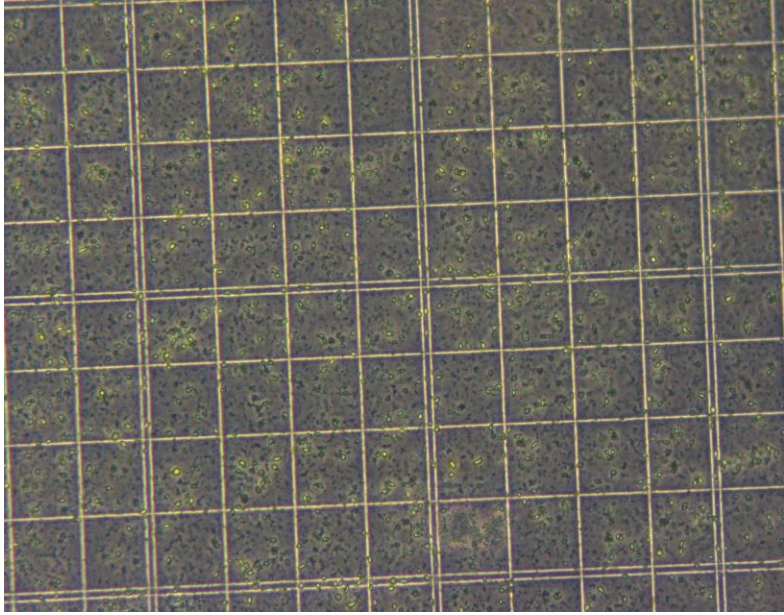
**Appendix D: Chloroplast Solution (20x)**



(Experiment 1)



(Experiments 2)

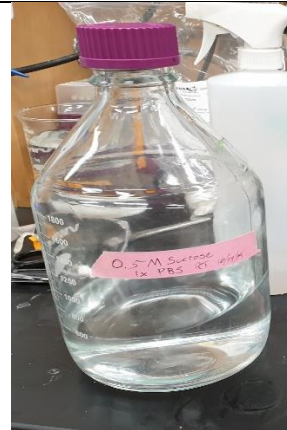


(Experiment 3)

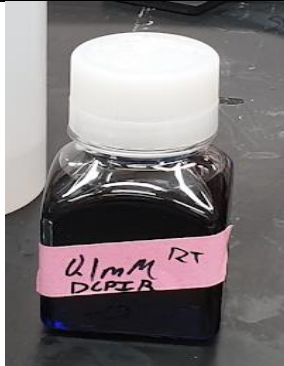
## Appendix E: Materials Needed



Spinach Leaves



PBS Solution



DCPIP Solution



Mortar and Pestle



Coffee Filter Sheets



Centrifuge





Micropipette



Glass Funnel



Centrifuge Tubes



Microcentrifuge Tubes