Topical probiotics for reducing infections by multidrug resistant bacteria

BME 301
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Table of Contents

Abstract

Lactobacillus rhamnosus GG (LGG), a probiotic widely used in various clinical fields, is found to be beneficial in enhancing body health condition. In order to research on the effectiveness of applying LGG to the interior nasal cavity to reduce *S. aureus* bacteria, a delivery device for LGG is needed to conduct clinical trials. There are three design choices for the delivery device, including the Dry Powder Nasal Spray, the Liquid Nasal Spray, and the Gel with Blister Pack Applicator. After the design evaluation process, the gel with the blister pack applicator design was chosen as our final design due to higher preferences of the client and average users along with its ability to better satisfy our design requirements. Testing in the future will be used to figure out a suitable gel to suspend the LGG in. Testing the viability of the bacteria, both in the gels and in the actual nasal cavity, is also necessary.

1.0 Introduction

1.1 Problem Statement

Our client, Dr. Nasia Safdar, of the UW-Madison Department of Medicine, researches the use of probiotics. Currently, she is researching the efficiency of the probiotic LGG in preventing *staphylococcus aureus* infections when the probiotics are applied to the interior nasal passage. A device to deliver the probiotic to the inside of the nose is needed to perform clinical trials with the probiotic. The delivery device should allow the accurate delivery of one billion viable lactobacillus gg organisms to the nose. Also, a solution in which to suspend and deliver the bacteria to the nose needs to be found. The lactobacillus gg should live inside the nasal passage for at least one day to allow for daily application of the probiotic.

1.2 Background

1.2.1 Probiotics

Probiotics, as defined by the World Health Organization, are "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host." Probiotics can be used to balance the immune system in two major ways. First, probiotics can be used to restore a balance of good bacteria to the body in cases where antibiotics used to kill unfriendly bacteria in the gut also killed those bacteria that are beneficial to the digestive and immune system. This death of beneficial bacteria may lead to side effects, such as cramping and diarrhea, which probiotics can be used to relieve. Second, when disease-causing bacteria, yeast, or other

unfriendly microorganisms invade the body, probiotics are able to stop or suppress the growth of these harmful microorganisms (National Center for Complementary and Alternative Medicine). LGG is a strain of bacteria and is one of the most common probiotics used in clinical applications. LGG has been proven to be effective to treat and prevent acute diarrhea and antibiotic-associated diarrhea. It is also suggested that LGG may prevent allergies, respiratory infections, dental caries, and nasal colonizations of potentially pathogenic bacteria such as *staphylococcus aureus* (*s. aureus*) and *streptococcus pneumonia* (Doron et al., 2005).

1.2.2 *Staphylococcus aureus*

S. aureus is a common type of bacteria that can be found in roughly 30% of the people's nostrils. Normally, s. *aureus* stays inside the nasal cavity and the person is considered "healthy." However, once *s. aureus* gets inside the body, they may cause minor infections of the skin like pimples and boils or serious infections, such as blood infection and pneumonia, which can be fatal (Center for Disease Control and Prevention). Methicillin-resistant *s. aureus* (MRSA) are s. *aureus* bacteria that are resistant to antibiotics and methicillin. Three LGG derived peptides, NPSRQERR, VHTAPK and PDENK, have been shown *in vitro* to affect the growth of MRSA bacteria (Lu, 2010). Instead of simply applying these peptides, our client hopes that establishing a colony of LGG in the most outer part of the nasal cavity will be able to prevent MRSA infections in those who have the bacteria present in their nasal cavities.

2.0 Motivation

S. aureus are bacteria that cause a multitude of infections including those of the skin and respiratory system. About 30% of people have these bacteria in their nasal cavity, but won't get sick from them under normal circumstances. However, if a person has a wound or a compromised immune system, these bacteria can significantly raise the risk of these people getting an infection. Antibiotics applied to the nasal cavity have been shown to eliminate the *s. aureus* bacteria, but the antibiotics are harmful to the useful bacteria in the body as well. Antibiotics applied over large areas or for long periods of time create resistant bacteria strains that can cause recurrent infections in the same person or in others. One example of a developed resistance is seen in MRSA, which are especially dangerous because the antibiotic, methicillin, is no longer effective to cure the infection. The economic burden of these bacteria on hospitals due to increased length of stay, more complications, and an increased risk of death ranges costs

patients a lot of money each year. In 2003, the total economic burden of *s. aureus* infection was estimated to be $14.5 billion for all inpatient stays and $12.3 billion for surgical patient stays (Noskin, 2007).

Our client is interested in controlling bacteria through probiotics. Our client is studying the effect of LGG on the baseline levels of staphylococcus flora in the nasal cavity. Probiotics have been studied orally, but since *s. aureus* resides mainly within the nasal passage, our client is performing clinical trials to test the efficiency of LGG in killing or reducing the number of harmful staphylococcus aureus when LGG are applied to the nasal passage. The client is exploring the use of probiotics over the current antibiotics that are on the market for killing the *s. aureus* in the nose. This is due to the fact that by using the probiotic bacteria LGG is that there is no danger of the harmful *s. aureus* developing a resistance to the LGG like it eventually would develop with antibiotics. Therefore, the LGG treatment could be possibly be used for long periods of time. The delivery device is needed to successfully complete the clinical trials to study the effects of LGG.

3.0 Design Specifications

3.1 Client Requirements

Figure 1.Culturelle capsules (Walgreens).

The ultimate goal of the project is to design a device for delivering the probiotic bacteria, LGG, to anterior nasal passage. The probiotics to be delivered are the bacteria LGG, with the trade name, Culturelle (Figure 1). The standardized amount for accurate delivery should be one billion organisms each time, and ideally the device should be used repeatedly for multiple deliveries over a short period of time. Each Culturelle capsule each contains 9 billion organisms, so the LGG in the capsule needs to be diluted or delivered in smaller amounts. The selected solution that suspends the bacteria within the delivery device should be biocompatible with the human body. The solution should also allow the bacteria to live for up to two weeks and prevent it from overgrowing while in storage. Once delivered, the bacteria must live in the nose for a minimum of one day in order to obtain the reliable observations of the impact that LGG living in the nasal cavity has on treating *s. aureus* infections. In addition, there should not be any food for the bacteria in the device.

The delivery device should be able to be refrigerated in order to keep the bacteria alive for up to two weeks. The materials should be able to withstand refrigerated storage conditions, where the temperature is around 4°C to 5°C. The device needs to be opaque to keep out light that would promote bacteria growth.

If insertion of the device into the nose is needed, the insertion should not be further than 1-2 cm into the nasal passage. Any insertion of the delivery device further into the nasal passage may result in the LGG solution traveling into the sinus cavities and throat, rather than solely into the nose. For the patients' convenience, the device should be easy to transport, with dimensions of less than 7 cm x 7 cm x 2 cm and a weight less than 0.25 kg. The materials for the delivery device must not induce any harm to any user.

3.2 Ethics

The purpose of this project is to design a device or method of delivering probiotics in order to investigate the impact of applying LGG in nasal cavity. Therefore, actual testing on human subjects is necessary for accuracy and practicality. While performing the experimental trials, the patient confidentiality should be maintained. The device will be clinically tested by our client following proper established guidelines for running such a test. Moreover, it is important to ensure that any input must not be harmful when testing on any human subjects or other living subjects. Substances, whose effects on humans are uncertain, may not be used on human subjects without prior testing that shows their safety when used on humans. Lastly, the product should be carefully designed and evaluated to prevent of unintentional copying other existing products on the market.

3.3 Ergonomics

The probiotic delivery device needs to keep LGG bacteria in storage for 2 weeks while delivering the bacteria to the anterior nasal passage in viable condition. It should not damage the bacteria or cause harm to the patient. The solution that the bacteria are mixed with should not cause the bacteria to grow or kill the bacteria. The exterior housing for the delivery device should be free of sharp edges so the user is not harmed. The forces applied by the user to the device should not be excessive.

4.0 Existing Devices

There are currently many nasal drugs on the market today; however, none of them use probiotic bacteria to treat *s. aureus* infections. Examples of existing devices include Afrin, Flownase, and Bactroban. There are many other types of nasal spray brands that solely deliver saline solutions to rinse the nasal cavity and moisturize dry noses.

4.1 Afrin

Afrin (Figure 2) is an over the counter nasal spray used to treat discomfort of the nose and sinus area caused by colds and sinus infections. It contains a drug that narrows the blood vessels around the nasal cavities. Although Afrin is relieves pain, it does not treat or cure any disease, it only removes the discomfort associated with symptoms, but it is not recommended for use longer than 3 days. Afrin comes as a liquid nasal spray (National Center for Biotechnology Information). The average cost of Afrin is approximately $7-$10 US dollars (Walgreens).

Figure 2.Afrin liquid nasal spray (Walgreens).

4.2 Flownase

A second product currently on the market that is similar to Afrin is Flownase (Figure 3). Flownase is a prescription corticosteroid that prevents swelling of the nasal tissue to relieve sneezing, itchiness, and stuffiness of the nose (National Center of Biotechnology Information). It is prescribed to treat the above symptoms in relation to allergic rhinitis and perennial non-allergic rhinitis. Like Afrin, Flownase is a liquid nasal spray. The liquid contains the corticosteroid. The average cost of Flownase is between $40 and $80 US dollars depending on insurance coverage (Walgreens).

Figure 3.Flownase liquid nasal spray (Walgreens).

4.3 Bactroban

Bactroban (Figure 4) is a prescription antibiotic ointment that is used to treat *s. aureus* infections. Bactroban is commonly used to treat impetigo, skin infections, and fungal infections. Bactroban is applied 2-3 times daily for 1-2 weeks onto the affected area, which is the nose in the case of *s. aureus* infections (National Center for Biotechnology Information). Bactroban nasal ointment has an average cost between $20 and $70 US dollars depending on insurance coverage (Walgreens).

Figure 4.Bactroban nasal ointment (Walgreens).

5.0 Design Proposal Overview

The delivery device will be used to deliver the probiotic bacteria, LGG, to the anterior nasal passage. Accurate delivery of one billion viable organisms is required for each dosage. The LGG when placed in the nasal passage will be used in clinical trials testing the efficiency of LGG in eradicating *s. aureus* from the nasal passage. The delivery device should not kill the bacteria or harm the patient. All three designs feature a different solution that the LGG bacteria are suspended in during delivery. Each design has different features that distinguishes itself and provides a unique solution to the problem. The survey results referenced in the designs can be found in Appendix C: Survey Results.

5.1 Design 1: Dry Powder Nasal Spray

The first design for delivering the probiotic, LGG, to the nasal cavity is using a dry powder nasal spray. The dry powder nasal spray would consist of the LGG in a powder form being sprayed into the nasal cavity. There would not be any liquid to accompany the powder, so the bacteria and the materials used to encapsulate the probiotic bacteria would land directly on the inside surface of patients' nasal cavity. See Figure 5 for a picture of how the dry powder nasal spray is delivered.

Figure 5.Dry powder nasal spray.

The Culturelle capsule contains the powder form of the LGG inside the capsule itself. In order to isolate the powder, the capsule needs to be opened and the powder form of the LGG needs to be poured into a spray bottle. The spray bottle is opaque to prevent light from reaching the LGG to prevent overgrowth. The bottle would also need to have a short (less than 2 cm long) nozzle to prevent the sprayed powder from traveling too far back in the nose. We want the powder to land inside the nose and not inside the sinus cavity. An example dosage requirement is that the patient would spray two sprays into each nostril to deliver the 1 billion LGG organisms. This requires that each spray of the dry powder would deliver 250 million LGG organisms.

There are several advantages and disadvantages of the dry powder nasal spray design option. A benefit of this design is that the powder spray would be easily carried out by patients since it is very similar to the delivery of liquid nasal sprays used commonly to treat allergies and sinus infections; the patients are familiar with the procedure of using the spray. Another benefit is that the bacteria would not have to be encased in anything, such as a liquid or gel that could

affect the growth and survival of the bacteria. The bacteria would reside inside a bottle as opposed to inside a capsule and this is how Culturelle's LGG bacteria reside and there is not an issue with storing the LGG this way for 2 weeks. A downside of this design is that the dry powder may irritate a person's nose and cause them to sneeze. In the process of sneezing the bacteria may be expelled from the nose, thus defeating the purpose of applying the powdered LGG in the first place. Another downside of the design is that dry powder is not a common method of delivery for drugs applied to the interior of the nose and patients would not be as familiar with the application process and therefore not as receptive applying the LGG. Only 14% of people surveyed chose the dry powder nasal spray as their optimal first choice of nasal treatment delivery.

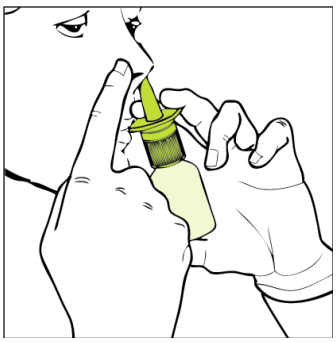

Figure 6.The procedure of using a liquid nasal spray.

5.2 Design 2: Liquid Nasal Spray

The second design for delivering the probiotic to the nasal cavity is using a liquid nasal spray (Fig. 6). The liquid nasal spray would consist of the LGG bacteria being dissolved within a 0.9 % saline solution. The liquid with the bacteria would be sprayed onto the inside of the nose.

Like in the dry powder nasal spray design, the liquid nasal spray will require that the capsule is opened and the powder form of the LGG bacteria will be mixed with the 0.9 % saline solution. The bottle still needs to be opaque to prevent the light from striking the LGG, which would promote the growth of the bacteria. A shorter nasal spray nozzle is needed if using this design to prevent the LGG from going too far into the nose and entering the sinus cavities. During the preliminary testing as described in Appendix B, it has been proven that LGG can last for at least three days alive without overgrowing within the 0.9% saline solution, which suggests that this design is plausible, although further testing for a week long storage period is needed.

The liquid nasal spray design has different advantages and disadvantages in using it. One of the advantages of using the liquid nasal spray is the ease of controlling the output amount per spray. The number of sprays needed per application would need to be relative to the concentration of LGG in the saline solution and the output volume per spray of the spray bottle. Another advantage of choosing this design is that more people are more familiar with liquid nasal sprays. According to the survey, the majority of subjects chose the liquid nasal spray as their second choice in nasal drug delivery based on personal preference of nasal drug delivery

methods. Although choosing the liquid nasal spray might be more user-friendly, there are some other drawbacks needing to be considered. In addition to controlling the delivered amount of LGG, the output distance might be too far, causing the solution to travel into the body, which does not serve the goal of the design.

5.3 Design 3: Gel with Blister Pack Applicator

The third design option to deliver the LGG bacteria to the nasal passage is a gel that is delivered in volumes placed inside a blister pack. The gel would contain the powdered LGG from the Culturelle capsule, suspended in the gel. Since the bacteria are thought to be most effective towards the most outer portion of the nasal passage, the gel will be applied close to the edge of the nose. This placement of the gel will prevent the LGG from dripping back into the nose. The gel will be sufficiently thick as to properly adhere to the interior surface of the nose. Preliminary testing showed that a gel would be comfortable and immobile after placement.

To deliver the correct amount of LGG (one billion organisms) a volume of gel with a specific concentration will be placed into blister packs (Figure 7), such as those that hold individual pills in a plastic casing and are opened by removing a thin metal film. The gel could be scooped out with a finger or a device such as a q-tip and put in the nose.

Figure 7.Blister pack similar to what the gel containing the LGG will be placed in.

The gel design has its own specific benefits and obstacles. Since most people are comfortable with using ointments, the gel would allow for a familiar application method to be used. Also, the gel formulation would be comfortable and stay in the location desired. The survey showed that the design ranked first the most often was the gel. An obstacle to producing a gel based probiotic solution is finding a gel that does not kill the LGG while suspending it within the gel cross links. Along those lines, the gel needs to be formulated with the correct consistency so that it does not drip or is too hard.

6.0 Design Evaluation

Table 1 below listed the categories that the team used to evaluate the three different designs. The final design would be chosen based on these criteria that include preference, accuracy, ease of use, maintaining bacteria viability, and precision of the device. The score in

each category is from 1 to 5, where 5 being the highest possible score. Then the total score would be added up from each category to determine the final design.

Table 1: Design Matrix for design evaluation

Categories	**Dry Powder Nasal Spray**	**Liquid Nasal Spray**	**Gel with Blister Pack Applicator**
Preference (5)	3	4	5
Accuracy (5)	3	3	5
Ease for use (5)	3	4	4
Bacteria viability (5)	5	4	3
Precision (5)	4	5	4
Total (25)	18	20	21

The first category is the preference of the user. The team conducted a small survey (see Appendix C) that asked people which design is the one that they feel most comfortable to use. The result showed that most people would choose gel as their first choice (41%), followed by liquid spray (35%) and then dry spray (14%). Plus, our client prefers the idea of using a gel-type approach, so the gel option score the highest point of 5.

The second category is the accuracy of the device to deliver most of the bacteria into the desired area, which is the front part of the nostril. Because most liquid sprays in the market (such as used for allergies) are designed to deliver the liquid deep into the nasal cavity, which is not desired by our client, using the liquid spray method may require special training or instructions. Moreover, it may be hard to aim the same area every time. The dry spray has the same kind of problem with liquid spray, so these two designs scored only 3 in this category. The gel can be easily applied to the front area of the nostril, so it scored a 5.

The ease of use of the device looks into the question of whether the design is easy to use by average patients. Since there are already lots of liquid sprays in the market, so this option would be the one that everyone is most familiar with. Thus liquid sprays scored the highest point in this category. Dry spray should operate the same way as the liquid spray, but since it may causes sneezing after use, so it only received a 3. The gel option, depending on the users and their preferences, may be unfamiliar to some of the people.

It is important that the bacteria could survive in the device for several days, because probiotics need live microorganism in order it to work. The dry spray has the advantage in this category because the source of LGG in our design is from the product Culturelle, which already has LGG in the dry powder form. And since the company of Culturelle claimed that LGG can

survive in this form for 2 weeks, the dry spray scored the highest point of 5 in bacteria viability. The team had conducted a small experiment that tested if LGG could survive in the saline solution (see Appendix B), and received positive results that LGG can maintain their viability in the solution for 3 days. Thus the option received a score of 4. With the gel option, since the team hasn't set up any experiment yet to test how well the bacteria would live in the gel-type formation, this design receives a 3 so far and could be changed in the future.

The precision of the device evaluates how precise the device can deliver 1 billion bacteria every single time. The liquid spray is probably the most precise device to deliver roughly 1 billion bacteria constantly, while dry spray may have the problem of bacteria being sneezed off and gel may be hard to apply at a consistent amount.

Based on the final scores of the three designs, the team has decided to choose gel option as the final design. However, since the bacteria viability in gel is not determined yet, the final design might be changed from gel to liquid spray since the two options received roughly the same score.

7.0 Future Work

The LGG will have to be proven to survive in the media of choice. A three day survivability test in 0.9 % saline was completed with promising results. Since we will be proceeding with the gel with a blister pack applicator, this experiment will have to be repeated. If they survive this preliminary test, the bacteria will be tested to see if they survive for a period of one week (the time of use). If they are able to survive this long without overgrowing and dying, then the storage conditions of two weeks will have to be conducted. If the bacteria don't survive, something like glucose will be added to the gel as a source of food. Saline may also have to be incorporated to obtain proper electrolyte levels. After survivability in the media is perfected, the concentration of bacteria will have to be altered so the number of bacteria that should be delivered is one billion. The concentration depends on the success of delivery and the preparation of the gel. The LGG living within the nose after period of time, will be tested by applying the media to a person's nose and swabbing the area at different time points to determine how many bacteria have survived after a specific number of hours, up to 24 hours. Then, our job is complete and the clinical testing of the bacterial device can begin.

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Appendix A:
Product Design Specification Report
Probiotics Delivery Device

Date: 26 February 2011

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Problem Statement

Our client, Dr. Nasia Safdar, of the UW-Madison Department of Medicine, researches the use of probiotics. Currently, she is researching the efficiency of the probiotic lactobacillus GG in preventing *s. aureus* infections when the probiotics are applied to the interior nasal passage. A device to deliver the probiotic to the inside of the nose is needed to perform clinical trials with the probiotic. The delivery device should allow the accurate delivery of one billion viable lactobacillus GG organisms to the nose. Also, a solution in which to suspend and deliver the bacteria to the nose needs to be found. The lactobacillus GG should live inside the nasal passage for at least one day to allow for daily application of the probiotic.

Client requirements

- Deliver probiotics to anterior nasal passage
- Probiotics to be delivered are the bacteria lactobacillus GG
    - Trade name: Cuturelle
- Accurate delivery of 1 billion organisms
    - Repeatability of delivered dose
- Solution needs to be found to suspend bacteria in
    - Biocompatible with human patients
    - Solution must allow bacteria to live for up to 2 weeks
    - Solution must keep bacteria from overgrowing
    - No food for bacteria will be present
- Once delivered the bacteria must live in the nose for a minimum of 1 day
    - Will allow daily delivery of probiotics by patient
- Delivery device will need to be able to be refrigerated
- Delivery device should be opaque to keep out light that would promote bacteria growth
- Weigh less than 0.25 kg
- Dimensions less than 7 cm x 7 cm x 2 cm
- Delivery device must prevent insertion of delivery device further than 1-2cm into the nasal passage
- Material of delivery device must not harm user
    - Non-abrasive
- Material must not degrade with constant use
    - Lifetime is 2 weeks
    - Use daily

- Material must withstand refrigerated storage conditions
    - 4-5°C
    - 50% humidity

Design requirements:

1. Physical and Operational Characteristics

a. *Performance requirements*: The device will be used daily to deliver the dosage of 1 billion bacteria. The device and bacteria suspended in a solution must allow the bacteria to survive for up to 2 weeks. The device must have ability to secure bacteria inside to prevent contamination of outside surfaces or of bacteria itself.

b. *Safety*: This device must not endanger the user. There must not be toxic materials or sharp edges within the device. There should not be any pathological concerns due to fluids escaping the delivery device. Neither the solution, nor the delivery device should cause harm to the patient.

c. *Accuracy and Reliability*: This device should accurately deliver 1 billion organisms. This delivery should be precise and repeatable for daily delivery up to 14 days. The solution should not cause the bacteria to die or grow excessively.

d. *Life in Service*: The device should have repeatable delivery procedures for 2 weeks. The materials should uphold their features to allow for multiple deliveries of probiotics. The solution should not allow excessive growth or death of bacteria for 2 weeks.

e. *Shelf Life*: The materials of the model should not degrade over time in refrigerated storage for 2 weeks. The solution should not allow bacteria to die within 2 weeks. The solution will be made a maximum of a week before the patient receives it. The patient will have it for up to a week for use; the total shelf life is 2 weeks.

f. *Operating Environment*: There will be one device per patient. The delivery will be performed at ambient conditions. The storage will be at 4-5 °C in a refrigerator.

g. *Ergonomics*: Delivery device should only be used to deliver the prescribed probiotics and should be discarded after use. The probiotics should be taken only in prescribed dose.

h. *Size*: The device should not exceed a size of 7 cm x 7 cm x 2 cm.

i. *Weight*: The delivery device with the probiotics suspended in the solution should weigh less 0.25 kg.

j. *Materials*: Materials must be safe for use with humans. Any material used should not pose a health risk. Non-radioactive, non-flammable, and non-corrosive materials should be used. Material must not degrade when introduced to the nasal passage. The solution must not be harmful to the bacteria or patient.

k. *Aesthetics, Appearance, and Finish*: The device should be pleasing to the eye. The finish should be smooth and clean looking.

2. Production Characteristics

a. *Quantity*: One device is required at this time. However, since the device is to be used on a large scale clinical trial, additional models should be able to be available.

b. *Target Product Cost*: The target manufacturing cost for the product is $10 per delivery device. This target cost includes the bacteria and the solution. The target cost is based on a mass production of the device; the first device will have a target cost of under $150 for the lab supplies, solutions, culture media, and bacteria (the lab is fully equipped and some culture media and solutions will be used from the current stock).

3. Miscellaneous

a. *Standards and Specifications*: This device will require approval by the FDA if this product with the delivery device is mass produced for market use after the clinical trials take place since it will be used with patients. Currently, the device falls under Class I classification and does not require any premarket notification to the FDA regarding the device.

b. *Customer*: The delivery device should adhere strictly to the basic requirements of delivering bacteria to the nasal passage of the patient. The device and the bacteria should be used as prescribed.

c. *Patient-related concerns*: The delivery device will come in contact with patients and therefore should not cause harm to the patient. The patient should not be made sick by the materials of the device or its probiotic contents. Patient confidentiality should be maintained while building and testing the delivery device.

d. *Competition*: There are many types of products that focus on delivering fluid solutions to the nose. Three examples are Afrin, Flownase, and Bactroban. There is not a marketed ointment or sprayer specifically for delivering probiotics to the nasal passage.

Appendix B: Lab Procedure and Results for Culturing Lactobacillus GG

We cultured the LGG in the lab to determine the number of organisms that were still living within a 0.9% saline solution at periods of 0 hours, 24 hours, and 48 hours after the Culturelle capsule contents were placed in the saline. To culture the LGG bacteria in the lab, we followed the procedure listed below (Gebreselassie, E.):

"1. Take 3 capsules of LGG and vortex mix the contents separately in a sterile saline solution (10 mL).
2. Dilute the suspension serially in 900μL sterile saline solution by transferring 100μL from the suspension.
3. Plate 100μL from dilutions 10^{-4}, 10^{-5}, and 10^{-6} on de Man Rogosa Sharpe (MRS) agar plates and keep the dilutions at 4°C to plate the next day.
4. Incubate plates in anaerobic jar for 48 hours at 37°C and count colonies.
5. Determine the initial number of bacteria in the Culturelle capsule by multiplying with the dilution factors.
6. After 24 hours, plate the same dilutions kept at 4°C, dilutions 10^{-2}, 10^{-4}, 10^{-6} on MRS agar plates and incubate the plates in anaerobic jar at 37° for 48 hours. Count the number of bacteria.
7. Repeat step 6 at 48 hours and see if there is a significant decline in the number of probiotic bacteria."

After completing the culturing and counting, we obtained results that showed that there was not a significant decline in the number of surviving bacteria after 3 days in the saline solution. The results are below. The A, B, and C represent the three different Culturelle capsules used in Figure 8.

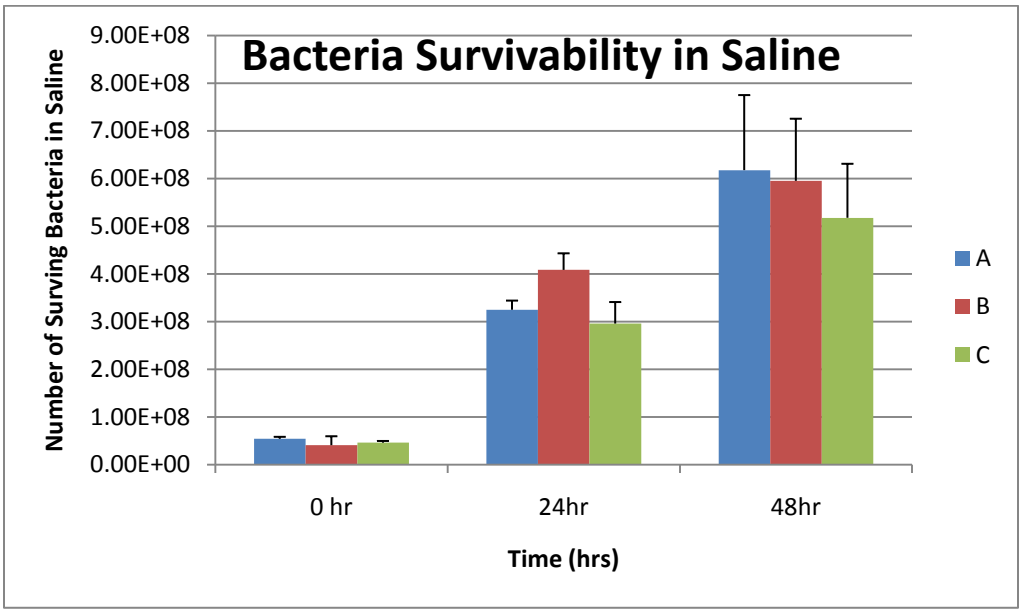

Figure 8. The bacteria were tested in a 0.9% saline solution kept at 4 degrees C at 0, 24, and 48 hours by plating different concentrations on LB plates and grown anaerobically. The plates which contained more than 30 and less than 200 colonies were counted and the initial concentration of colony forming units per milliliter was determined. The bacteria proliferate a significant amount during this time, but not greater than an order of magnitude.

Appendix C: Survey Results

To help the group to decide which design we should proceed, we conducted a survey that asked people's opinions on which types of delivery method would be the one that they feel most comfortable to deal with. The participants were mostly composed of the group members' friends or families. The survey had the participants ranked their preferences from which one is the most desired method to use to the least desired method. See (Figure 9 and Table 2) survey results in the table and figure below. Interestingly, most people chose the gel method (41%) or the liquid spray (35%) as their number one option. But on the other hand, many other people didn't like the concept of applying gel to their nose (30%). Liquid drop option was generally the people's last two choices, and the dry spray was not chosen by a lot of people to be their first choice too. Since the gel method received the most 1st choice vote, the team decided to develop the gel method first.

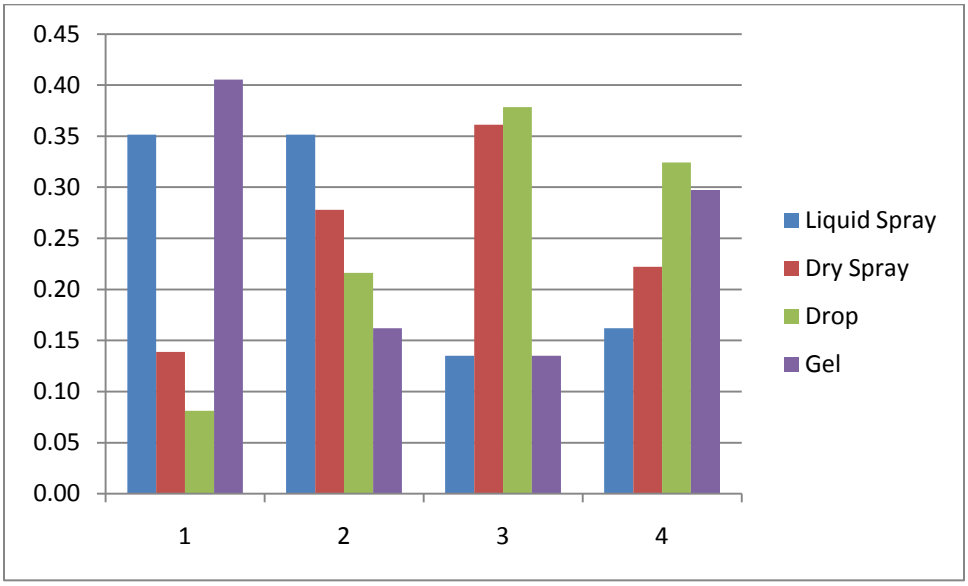

Figure 9. Survey results in chart form.

Table 2. Table form of results of survey.

	1	2	3	4
Liquid Spray	0.35	0.35	0.14	0.16
Dry Spray	0.14	0.28	0.36	0.22
Drop	0.08	0.22	0.38	0.32
Gel	0.41	0.16	0.14	0.30