Device for Converting Elastin-Like Polypeptide into Soluble Form

March 3, 2006

Team Members:
Dhaval Desai (Team Leader)
Lee Linstroth (Communicator)
Malini Soundarrajan (BSAC)
Nathan Kleinhans (BWIG)

Client:
Dr. Darin Furgeson Ph.D.
School of Pharmacy
University of Wisconsin – Madison

Advisor:
Dr. Naomi Chesler Ph.D.
Department of Biomedical Engineering
University of Wisconsin – Madison

ABSTRACT

Cancer is a disease that has affected many people worldwide. Current treatments for cancer include chemotherapy, radiation therapy, hormonal therapy, and immunotherapy. These treatments are invasive and also harm normal tissue. Less invasive and tissue specific targeting treatment options are to be sought. Elastin-like polypeptides (ELPs) may serve as a gene and drug carrier. ELPs are ideal carrier agents for cancer treatments because they are hydrophobic, undergo inverse state transition, and are non-immunogenic; however, the hydrophobicity of ELPs collapse at a transition temperature (T_t) and they aggregate. This transition is reversible. A device is needed to convert ELP aggregate back into a soluble form so research on ELPs can be conducted. This device will require minimal manual labor and will resolubilize ELP rapidly.

PROJECT STATEMENT

Cancer is a condition characterized by uncontrolled cell growth. Current treatments for cancer include chemotherapy, radiation therapy, and hormonal therapy. These approaches are invasive and also target normal cells. Elastin-Like Polypeptides (ELPs) may serve as promising gene and drug-delivery agents to treat various types of cancers. After harvesting ELP from bacteria, ELP becomes aggregated. For our client, Dr. Furgeson, to conduct research on ELPs, soluble form is required. The purpose of this project is to design a device that will make it easy to convert ELP aggregate into soluble form.

INTRODUCTION

It is projected that cancer will affect approximately 1.4 million individuals in the United States in 2006. This condition involves uncontrolled cell growth and diminishes the body's immune response. There are several types of cancers known – breast, lung, prostate, kidney, skin, and bladder. Genes, age, and environmental factors (smoking and alcohol) are the primary risk factors for cancer. Of the current treatment options, chemotherapy, radiation therapy, and hormonal therapy, usually two or more of these treatments are needed. [1]

Chemotherapy uses chemicals that specifically target reproducing cells to prevent cell growth ultimately to destroy the tumor. If complete eradication is not possible, chemotherapy strives to contain the spread of a tumor; however, if containment is no longer possible, chemicals work to alleviate cancer symptoms including fever, fatigue, and pain. [1]

Radiation therapy utilizes ionizing radiation, either photons or particle radiation, to penetrate through deep tissues of the body where reproducing cancer cells often reside. Also, the source of radiation can be external or internal. This radiation is capable of either destroying the actual cells or altering the genetic information of those cells. [1]

Hormonal therapy is widely used to fight breast cancer and prostate cancer. Estrogen and androgen are two hormones that manipulate the cell cycle in breast and prostate respectively. Hormonal therapy is carried out by either physically removing the hormone producing organs (ovaries and testis) or by

inhibiting hormone function in cell proliferation by introducing materials that antagonize the hormone's function. [2]

There are several detrimental effects of these cancer treatments. Chemotherapy and radiation therapy also target normally dividing cells. Hormonal therapy is a very effective technique for blocking hormone interaction in target tissues; however, tissues may not function properly with a hormone deficiency. For example, surgically removing ovaries from a woman with breast cancer will decrease estrogen production and thereby decrease the rate at which the tumor grows or even stunt the tumor growth. Consequently, this will adversely affect areas such as the bones and liver which require estrogen to properly function. Hence, a treatment option that offers minimal loss of normal cells is desired. [1, 2]

Advances in molecular biology have introduced the potential of incorporating actual fragments of DNA into a cell's genome to restore a missing function or give the cell a new function. Viruses and non-viral gene agents can be used to deliver a piece of DNA into a target gene. Although the transfection (introduction of foreign materials into the cell) rates are higher for the viral model, the non-viral model is preferred. Elastin-Like Polypeptides (ELPs) may serve as the non-viral mode gene-carrier.

ELPs are synthetic polymers that consist of repeated pentapeptide chain of amino acids. The five polypeptide sequence is: Valine – Proline – Glycine – X – Glycine (Figure 1). X is any amino acid except Proline. By changing X, several different forms of ELP can be synthesized.

ELPs are non-immunogenic, undergo inverse temperature transition, and are hydrophobic. When ELPs are introduced in the body, the body does not initiate an immune response against it.

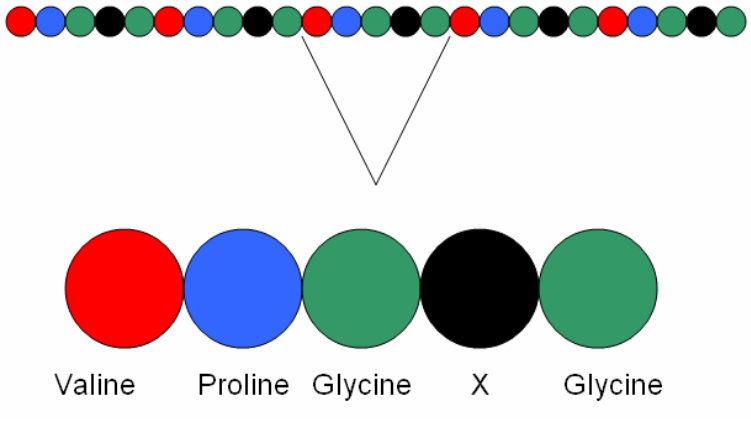

Figure 1: The basic structure of ELPs.

Every ELP molecule has a Transition Temperature (T_t) [5]. When the temperature of ELP goes above the T_t, the ELP becomes aggregated. When the temperature is below T_t, the ELP becomes soluble [3]. This property can aid with tissue-specific gene delivery. For example, an external heat source can increase the temperature of a tumor. When ELP encounters these cells, it will aggregate. Then, the cells would accept the DNA by endocytosis [4]. Lastly, the structure of ELP makes it hydrophobic, which determines transition temperature. By altering the "X" amino acid, many different combinations of ELPs can be synthesized with different transition temperatures. This approach is minimally invasive, tissue-specific, and non-immunogenic. Currently, our client uses bacteria to synthesize ELPs.

Several steps need to be carried out before ELP can be isolated from the bacteria. First, a vector with the ELP gene has to be genetically engineered. This vector is transformed into *E. coli* cells. These cells are then grown in Lauria Broth (LB) media. The cells increase in numbers while continuously transcribing

and translating the ELP gene into ELP polypeptide. After a certain amount of time, the cells are isolated and lysed. The ELP is separated from the endogenous DNA and proteins of the bacteria. The end product is an ELP pellet that is very viscous. The purpose of our project is to design a device that would help resolubilize this pellet in Phosphate Buffered Saline (PBS) solution. (Figure 2)

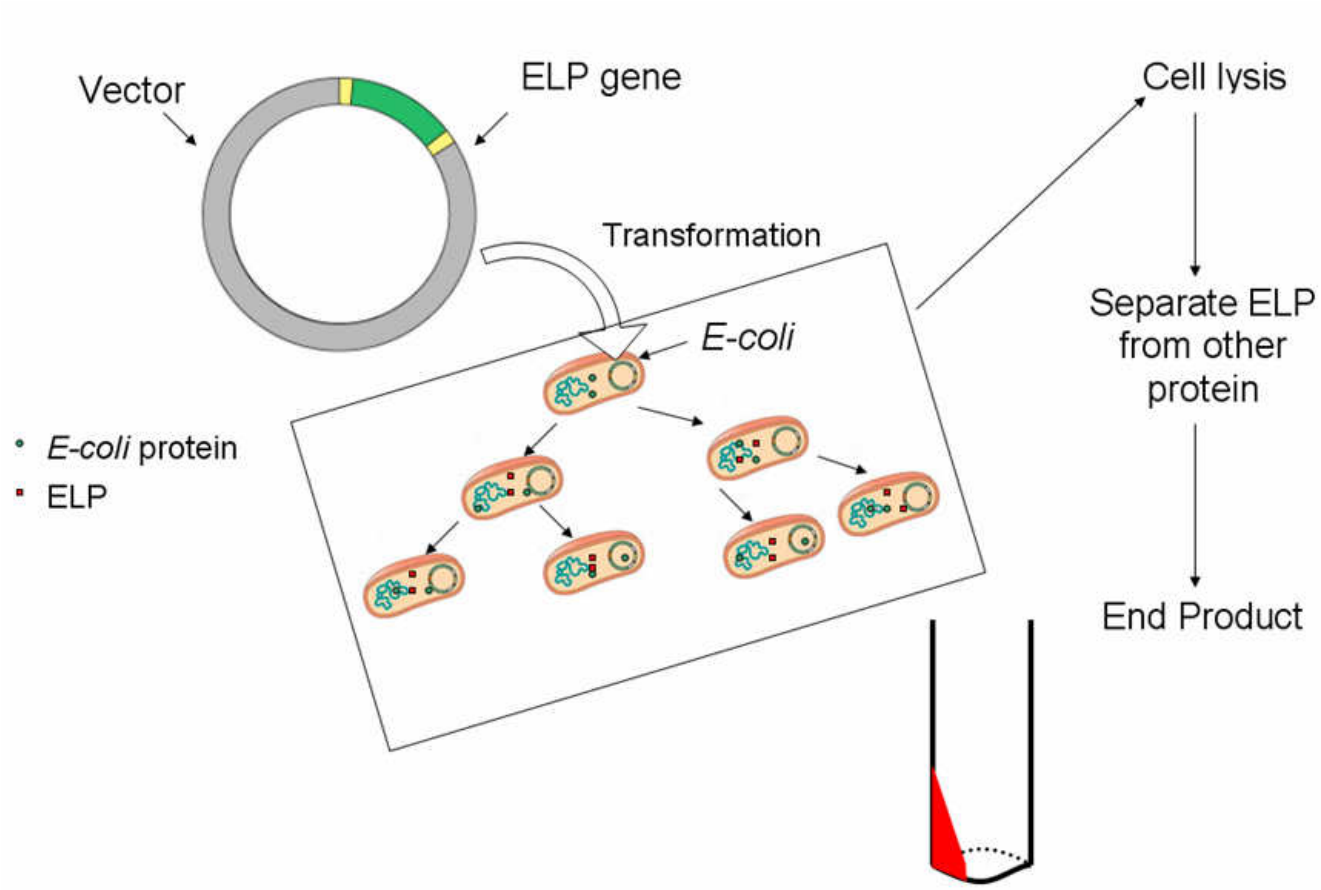

Figure 2: Current procedure used to isolate ELP. The red pellet is the viscous ELP pellet isolated at the end of the procedure.

DESIGN CONSTRAINTS

Our main goal in designing the device is to reduce the time required to solubilize ELP and to reduce product loss. The device should also use PBS solution as the solvent to solubilize ELP. Since ELP aggregates above the transition temperature, T_t, overheating in the device could be problematic; hence, maintaining low temperature is critical in keeping ELP from aggregating. A low temperature can be maintained by using the device in an ice bath. Therefore, the device should be able to operate within the temperature range 2^o to 30^oCelsius. Moreover, the device needs to reduce the amount of manual labor involved; the current process being used, repeated pipetting, requires several hours of labor, therefore, designing an automated device would benefit our client tremendously. Designing a device that is easily cleaned would also reduce the manual labor required to operate the device. The final device should resolubilize ELP harvested from 4 liters of bacteria (1 liter of bacteria yields approximately 180 mg of ELP). In addition, in the interest of safety, the device should have no exposed sharp edges that could harm the user. Precision and accuracy are also key components of this design. The final ELP solution should be uniform in concentration and at the end of each cycle, the concentration should be the same. Finally, the device should function normally for at least 50 cycles (1 cycle is equivalent to resolubilizing ELP harvested from 4 liters of bacteria at a time). Since the device will be on a lab bench top, it should fit in should fit in a 1 meter x 1 meter area; the height should not exceed 0.5 meters. Though the device does

not need to be portable, the device should weigh less than 20 pounds so that any lab assistant can transport the device with ease.

AVAILABLE PRODUCTS

There are many available products that mix the contents of test tubes, but none are specifically for resolublizing aggregates. Chemistry supply companies offer vortexers, rockers, and shakers, but none are vigorous enough to break apart ELP aggregate. All of these work by fixing the test tube to an external structure, rather than physically breaking apart the amassed ELP. Another major drawback to these is cost – most of this equipment costs upwards of $1500.

DESIGN ALTERNATIVES

Helix Model

This design would involve two intertwining spirals that form a helix. The helix would be cross-braced by blades or bars that will facilitate the majority of the resolublization process of aggregate. The helix will be formed to fit inside the test tube used to centrifuge the aggregate, without touching the walls of the test tube to avoid damage (Figure 3).

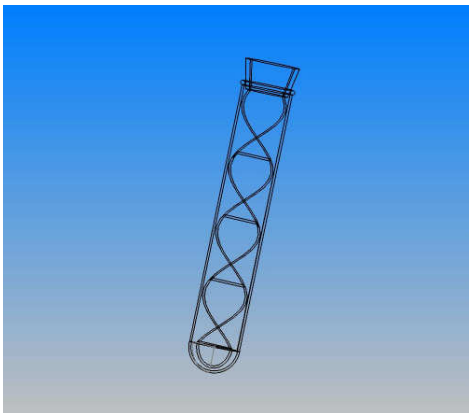

Figure 3: Wireframe model of Helix design inside test tube.

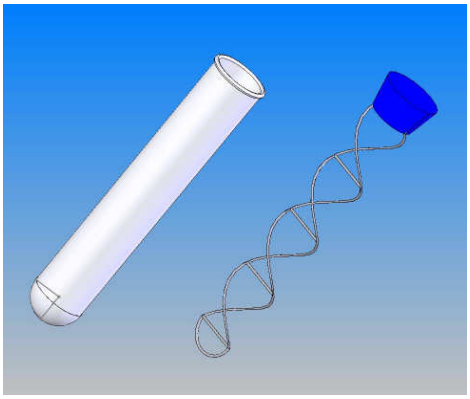

Figure 4: 3-D rendition of Helix model next to test tube.

The helix portion will be fixed to a rubber stopper, yet allowed to rotate independently (Figure 4). An external motor will be used to rotate the helix component at high speeds (upwards of 500 revolutions per minute), effectively mixing the aggregate with the PBS.

Advantages and Disadvantages

The advantage of this design is its versatility. The helix is easily cleaned and sanitized after each use, while the direct attachment to the motor allows for us to easily construct a circuit to allow adjustable speed. This design is also very cost effective, and resembles current industrial mixers.

Disadvantages include the possibility of a difficult manufacturing process. Since the helix will most likely need to be comprised of metal wire, welding is not an option, and we would need to epoxy or solder the blades in. The blades, if sharpened, could pose to be a safety hazard if the user is not careful. Since the helix is not meant to touch the sides of the test tube, this model might produce a low product yield.

Brush Model

This model consists of three distinct parts: the plastic centrifuge tube, a commercially available test tube brush, a lid for the centrifuge tube, and a motor to power the device. A hole through the lid allows the stem of the brush to fit into the centrifuge tube. The bristles of the brush are attached to a threaded rod; the brush will be 35 mm wide in diameter and 20 mm high. The stem that is attached

to the brush will be 150 mm in length. The dimensions of the brush were determined based on the dimensions of the test tube so that the brush is well-fitted to effectively resolubilize ELP.

On top of the lid, a small motor is connected to the rod of the brush and allows the brush to rotate to solubilize the ELP sample. The motor could either be powered by a battery or through AC current by plugging into an outlet. When the motor is powered, the brush rapidly rotates around the vertical axis and scrapes off ELP attached to the sides of the tube. One of the strengths to this design is the high surface area exposure of the bristles to ELP and PBS, which allow for faster and more efficient mixing. Since the bristles fit tightly against the centrifuge

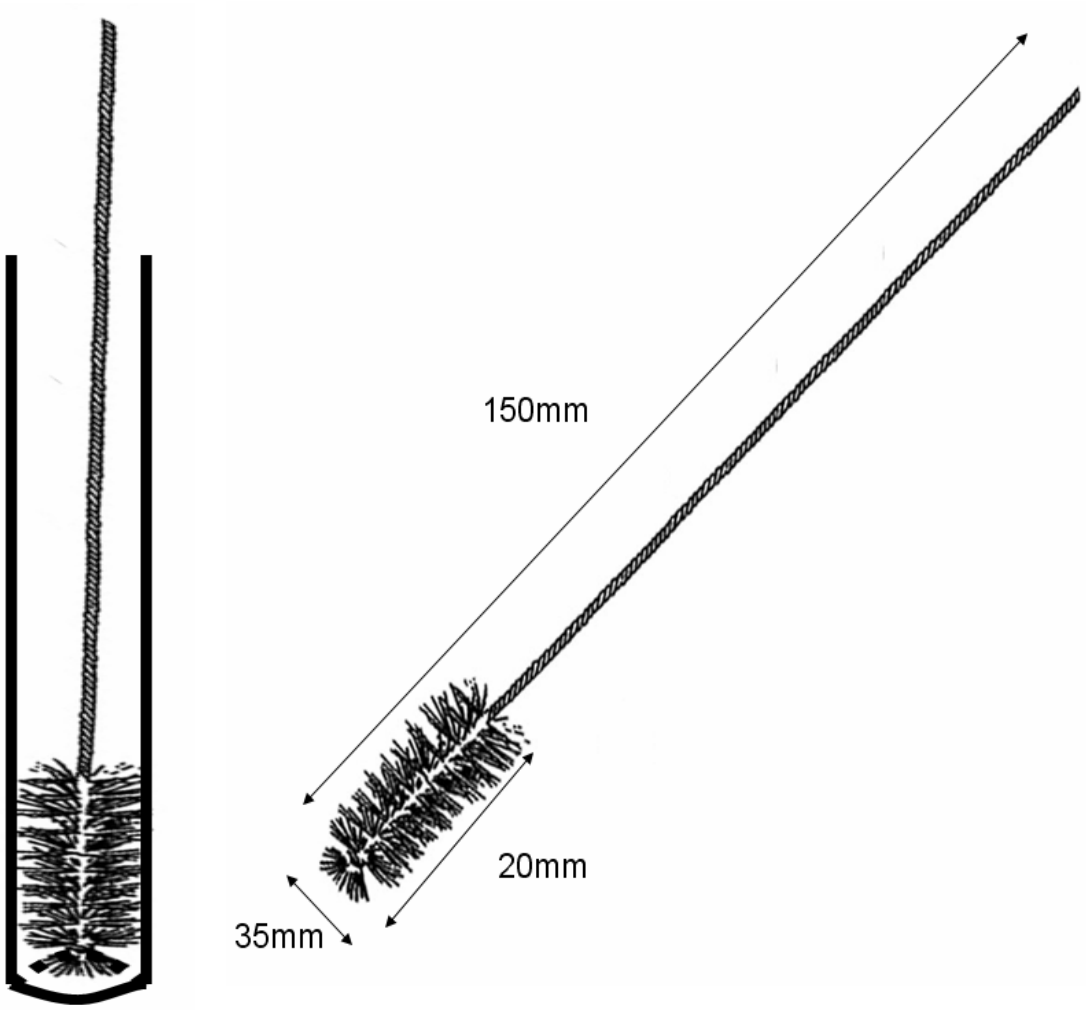

Figure 5: Brush model a) in test tube and b) with approximate dimensions.

tube, ELP aggregate will be scraped off of the sides and will get incorporated into the PBS solution. This feature is especially important since the ELP will be centrifuged before solubilization, and will therefore be attached to one side of the test tube.

Advantages and Disadvantages

Fabricating the device could be relatively simple since most of the parts are readily available in the market; however, we would still need to make adjustments to the brush to customize fit in the centrifuge tube provided by our client. While this model has some advantages such as simple fabrication, the test tube brush model also has several weaknesses. First, the bristles of the brush may be difficult to clean after use, this may also lead to reduced product yield. Since one of our client's requirements was to minimize product loss, this could potentially be a problem. On the other hand, if the ELP aggregate turns into a thin consistency similar to that of water or milk after solubilization, cleaning and product loss may not be problematic. Moreover, it is uncertain whether forces applied by the bristles would be enough to convert the ELP aggregate into a soluble form. In addition, the bristles of the brush are made of plastic and may wear out easily after use. Overall, the design has several positive aspects as well as drawbacks; however, more experimental work is required to get a better idea for the amount of force required to solubilize ELP.

Dual Scraper Model

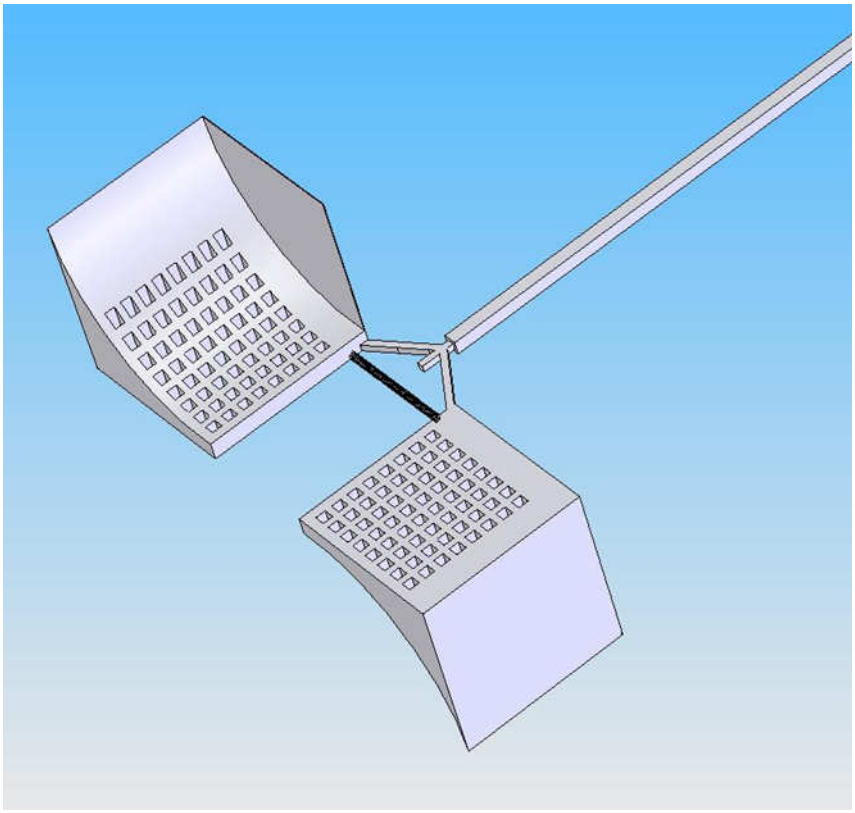

Figure 6 The dual scraper model consists of two scrapers to scrape the ELP from the inside surface of the centrifuge tube.

The Dual Scraper model (Figure 6) consists of two scrapers that are designed to scrape the ELP aggregate from the surface of the test tube and mix this aggregate with PBS solution. Since the opening of the test tube and the diameter of the bottom of the test tube have different dimensions, 18mm and 29 mm respectively, the scrapers must be able to collapse to enter the test tube and extend to the edges once inside. Therefore, the overall width of the collapsed scrapers must be less than 18mm and they must extend to approximately 28.5mm in order to reach the ELP on the side of the tube. This device also must have a mechanism external to the test tube to collapse the scrapers so that they can be removed from the test tube. These scrapers will rotate about a central axis powered by an external variable speed AC motor. Variable speed is ideal for this device because it will allow us to determine a speed that will effectively mix the solution but will not produce a lot of bubbles as this often results in product loss. The scrapers will be coated in Teflon or an alternative non-stick material to prevent the ELP from

adhering to their surface which will allow easy clean up. They will also have holes to allow ELP to pass through them as they are mixed into the solution.

Advantages and Disadvantages

This model will help spread apart the ELP allowing for more surface area to come in contact with PBS, which will rapidly increase the rate of solublization. The holes will also decrease the resistance on the scrapers thereby putting less strain on the motor. One drawback to this design is its difficulty to manufacture.

Design Matrix (see Appendix B)

We constructed a design matrix to determine which of our three alternative designs we were going to choose for the final design. The matrix scored five categories on a scale of 1-10 for each, with 1 being poor and 10 being the most desirable. The matrix itself is located in Appendix B. In summary, the dual scraper model scored the highest overall, with top scores in product yield and prototype life.

FUTURE WORK

After finalizing a design idea our team will determine what materials are needed for the device. We will construct a rough prototype that will allow us to perform multiple tests to evaluate its strengths and weaknesses as a mixing device. One test that could be done is to observe how efficiently the device can mix honey with food coloring. This will help us determine if the model mixes the contents uniformly and how fast it can do so. Following these tests, we will

modify the machine to improve problem areas and then retest. We will continue this process until we have a prototype that performs the desired actions at an acceptable level.

REFERENCES

1. American Cancer Society. 02/06/2006. Copyright 2006. Retrieved on 02/17/2006 from <www.cancer.org>
2. The Cleveland Clinic Taussig Cancer Center. 02/22/2002. Copyright 2003. Retrieved on 02/17/2006 from <www.clevelandclinic.org/cancer>
3. Betre H, Setton LA, Meyer DE, Chilkoti A. Characterization for genetically engineered elastin-like polypeptide for cartilaginous tissue repair. *Biomacromolecules*. 2002 Sep-Oct, 3(5): 910-6.
4. Chilkoti A, Dreher MR, Meyer DE, Design of thermally responsive recombinant polypeptide carriers for targeted drug delivery. *Adv. Drug Deliv Rev*. 2002 Oct 18; 54(8): 1093-111. Review
5. Chilkoti A, Dreher MR, Meyer DE, Raucher D. Targeted drug delivery by thermally responsive polymers. *Adv Drug Deliv. Rev*. 2002 Sep 13; 54(5): 613-30. Review

Appendix A – Product Design Specifications

Title: Converting Elastin-Like Polypeptide (ELP) aggregate into soluble form

Team:

Dhaval Desai – Team Leader
Lee Linstroth – Communicator
Malini Soundarrajan – BSAC
Nathan Kleinhans – BWIG

Date: February 1st, 2006

Function: Elastin-Like Polypeptides (ELPs) may serve as promising drug-delivery agents to treat various types of cancers. After harvesting ELPs from bacteria, ELPs become aggregated. In order for our client, Dr. Furgeson, to conduct research on ELPs, soluble form of ELP is required. ELP aggregation depends on temperature and salt concentration. Our device should efficiently convert ELP aggregate to soluble form.

Client Requirements:

- Device should be automated and require minimal manual labor.
- At least 80% of ELP should be recovered after it is converted into a soluble form.
- PBS should be used as the solvent in minimal amounts.

Design Requirements:

1. Physical and Operational Characteristics

    a) *Performance Requirements*
    The design should be able to resolubilize ELP harvested from 4 liters of bacteria (1 liter of bacteria yields approximately 180 mg of ELP).

    b) *Safety*
    The device should have no exposed sharp edges that harm the user. Any moving parts should be shielded to prevent the user from harm and all electrical elements should be contained to prevent unwanted shock or contact.

    c) Accuracy and reliability

The final ELP solution should be uniform in concentration with complete resolublization. Concentration uniformity will be measured by a spectrophotometer three times to verify concentration. Complete resolublization will be measured visually with total rehydration of aggregate pellet.

d) Life in service

The device should function normally for a minimum of 5 years if kept in optimal conditions.

e) *Operating Environment*

Device will be used in a normal laboratory setting.

- Normal room temperature operation (~22°C) for the majority of product life, but should continue to function normally within temperature range of 2°C to 30°C.
- Normal pressure ("the standard atmosphere" (1 atm) = 101.325 kPa)
- Low humidity
- Dirt and dust levels are low and negligible
- Fluid corrosion will may be a factor, as the laboratory setting may produce volatile fluid that could affect the product
- Vibrations from device or other equipment may cause loosening and detachment of parts and should be inspected regularly

f) *Ergonomics*

The device should be user friendly and easy to clean. Sterile equipment is a must, so design should allow for easy and quick cleanup to be ready for multiple batches. The device should be easy to hold (if handheld) and not require extraneous movement to operate.

g) *Size*

The device should fit in a 1 meter x 1 meter laboratory bench top. The height of the device should not exceed 0.5 meters.

h) *Weight*

The weight of the device should not exceed 20 pounds.

i) *Materials*

The materials should withstand the temperature range mentioned earlier in this PDS. The materials should withstand forces applied to it from motion, test tubes, or ELP. For example, if a motor is incorporated, it should be powerful enough to withstand any forces applied to the attached mechanism.

j) *Aesthetics, Appearance, and Finish*

The chamber, in which the ELP solution will be made, should be transparent. Product is not required to be aesthetically pleasing as long as functional conditions are met.

2. Production Characteristics
    a) *Quantity*
    Only one unit is desired.

    b) *Target Product Cost*
    The total product cost should be less than $100.

3. Miscellaneous
    a) *Customer*
    Our client prefers that the resolubilization process takes less than 3 hours. Also, the client would like the final product to be as concentrated as possible.

    b) *Competition*

Many kinds of laboratory mixers, shakers, emulsifiers, and vortexers are available though equipment supply companies including Bionexus, Diager, and IncubatorShaker. This equipment is not currently ELP specific, and costs anywhere from $200 to $2500 each.

Appendix B – Design Matrix

Criteria	Brush Model	Helix Model	Dual Scraper Model
Ease of Cleaning	2	7	6
Product Yield	2	5	7
Cost	8	6	5
Ease of Manufacturing	8	4	7
Prototype Life	2	6	8
Total	**22**	**28**	**33**

Scores based on a 1-10 scale, with 1 being poor and 10 being highly desirable.