Device for Converting Elastin-Like Polypeptide Aggregate to Soluble Form

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Introduction

The goal for our project this semester is to develop a device that will enhance the solubility of Elastin-like polypeptide (ELP) aggregate. The device will incorporate temperature control, salt extraction, and particle size reduction capabilities. In addition, the device will be engineered in ways that it is semi-automatic and requires minimal labor to operate.

The motivation of the ELP project lies within our client’s research in cancer treatments. Currently, chemotherapy is one of the most effective cancer treatments available; however, the drug delivery system in chemotherapy is non-specific. Chemotherapy injects toxic chemicals into the blood stream, and the chemicals are carried throughout the body affecting both the cancer cells and normal cells. Chemotherapy patients are in a constant state of nausea, they are sick and listless, and they generally lose his/her hair. Our client, Dr. Furgeson, is researching possible drug delivery system with Elastin-like polypeptide, such that the cancer treatment will be cell specific and non-viral.

Elastin-like polypeptides are artificial biopolymers that are thermal responsive. Below a characteristic transition temperature (Tt), ELPs are soluble in isotonic solution, but when the temperature is raised above the Tt, ELP hydrophobically collapses and aggregates. Interestingly, depending on the conditions, the transition of ELP between its soluble and aggregate state is totally reversible.

ELP is a promising cancer drug delivery carrier due to its thermal responsive characteristic. Research is being conducted to tag the cancer drugs with the Elastin-like polypeptide such that the drug can be delivered to targeted cells. By raising the
temperature around the targeted cells via heat pad or infrared, the ELP aggregates in regions with temperatures above $T_t$. The targeted cells engulf the aggregate surrounding it by endocytosis. Once inside the cell, the drug-ELP complex is sent to lysosome (digestive organelle) for proteolytic cleavage of the polypeptide. The cancer treatment is harmless while it is in the drug-ELP complex, however, once the ELP is digested by the lysosome, the drug is administered to the targeted cells and acts to terminate the cellular activities.

**Current Method of ELP Extraction**

The current process for obtaining elastin-like protein is to first transform the gene which encodes for ELP into a bacteria plasmid. The bacteria would synthesize the specific ELP in large quantities according to the transformed gene. Once ample amount of ELP is grown within the bacterial cells, the bacterial cells are put through sonication. The sonication breaks down the cell membrane and organelles, releasing ELP into the containing solution. Extraction of ELP is done by separating the broken cell organelles from ELP by differential centrifugation. Once finished, the pellets (containing organelles) are discarded. The ELP is rinsed with PBS (phosphate buffered saline) and then placed in protein disulfide isomerase until ELP aggregates. With another cycle of differential centrifugation, further purification is achieved because the insoluble ELP aggregates are contained in the pellet and the supernatant is discarded. After purification, the ELP needs to be resolubilized. The current method is to first extract salts with ITC (inverse transition cycling). Salts lowers the transition temperature so in extracting them the transition temperature will go up and resuspension will be simpler. The next step is to pipette the ELP aggregate with cold PBS baths and continuously do this until the ELP is
resuspended. A one liter culture of the bacteria typically creates 50 -100 mg of ELP, resuspending 300-600 mg will take 12 hours with the current method of continuous pipetting. This process is time consuming and ergonomically unsound because of the continuous repetition.

**Design Specifications**

The device should be able to completely automate or partially automate the resolubilization of the ELP in cold PBS solution. It should be able to recover 75 – 80% of the ELP synthesized by the bacteria. The product should be able to resolubilize at least 300 – 600 mg of the ELP in 12 hrs, which is the current rate of manual resolubilization. Temperature of the ELP material should be maintained below Tt, which may be 5°C – 75°C depending on the ELP composition and concentration (Urry, Dan W.). Construction should be of durable materials that can withstand the reduced temperatures and allow for heat transfer to cooling surroundings for temperature control. Particle size of the ELP pellet material should be reduced to maximize contact area between the ELP and the PBS solution. The device should not be too heavy and if so desired should be easily transportable by one person. It should be able to fit within the confines of a laboratory bench top or fume hood and should be secured on top of a surface to prevent slippage.

**Proposed Designs**

To transform ELP to its soluble form, we will manipulate three principle factors that affect ELP solubility: salt concentration, temperature, and particle size. Salt extraction will increase the transition temperature which will make the temperature control aspect of the mechanism easier to deal with because the PBS bath will not need to
be cooled as much as if there was salt in the solution. Particle size reduction will allow the ELP aggregate’s surface area to increase, this will cause more contact with the chilled PBS solution, increasing solubility in a more efficient form. There are five main forms of particle size reduction; crushing, grinding, pulverizing, homogenizing, and blending. Crushing and grinding are typically done with solids, crushing can be done with crystal-like structures, grinding needs to be in a solid form, typically crystal-like form. Pulverizing is the use of sonication or other such methods where the desired particles that need to be reduced can be broken with vibrations, such as cell organelles. Homogenization is the constant process of mixing and spreading the desired material throughout a liquid and eventually mixing it thoroughly to reduce the initial concentration. Blending is like homogenization but with more force and it is done more quickly. Our assumptions throughout this process will be that mechanical stress will not affect the ELP because of its elastin-like properties. So shearing or blending will not denature it in any form. Also heat fluctuation, either extreme cold or extreme heat (much higher then transition temperature) will not cause denaturing and if it does the ELP will come back together once the environment is brought back to a more ELP friendly temperature.
Design One:

Figure 1: Section view drawing of first proposed design.

Design #1 incorporates the grinding principle of particle size reduction and a dry-ice bath for temperature control. In this design, there is a funnel into which the pellet material containing the ELP is added. Surrounding this funnel is a cylindrical container holding dry-ice. The dry-ice is much colder than the pellet material and therefore heat will transfer from the pellet material through the funnel and to the dry-ice, thereby cooling the pellet material until it is frozen. There is a cone-shaped drill that is attached to a motor. This drill will rotate and descend simultaneously to grind the frozen pellet material and along with gravity push the pellet material downward into a cylindrical non-stick tube. The rigid cylindrical non-stick tube leads from the funnel to a reservoir. This reservoir contains chilled phosphate buffered saline (PBS) solution which acts as a solvent and is maintained at a cool temperature by means of a cooling medium external to
the reservoir. This reservoir incorporates an inlet for the PBS solution, which also acts as an outlet for vapor. Grinding of the pellet material is done to reduce particle size and therefore increase contact area between the pellet material and the PBS solution. As the ground pellet material drops into the PBS reservoir, it will dissolve with more ease due to the decreased temperature and the reduced particle size.

An advantage of this design is that the frozen ELP is easier to grind than the semi-solid pellet material, which would most likely stick to the drill and block the opening of the non-stick tube. Another advantage is that the use of a dry-ice external to the funnel negates any heat generated by the drill. Some disadvantages include that the use of a motor would require electric power and that there are multiple components needing cooling namely, the funnel and the reservoir of PBS.

Design Two:

![Figure 2: Drawing of second proposed design.](image-url)
Our second design is much like our first design in that they both deal with breaking down frozen ELP into a powder. The ELP aggregate will be brought to -78 °C and frozen solid. We will then add the solid ELP into our device. The ELP is dropped down a cylinder whose bottom is a rotating metal disk containing blades, much like that of a cheese grater. A plunger of some sort will then be inserted down the cylinder and will press the frozen ELP through the grater. The ELP will be shaved into small shavings and will fall down into a churning bath of PBS where it will be dissolved.

One advantage of this design is the fact that it requires much less force break down the ELP into a powder. In design one, the frozen ELP was ground which could end up requiring a great amount of force relative to the size of the device. In design two, we will have a rotating disk with blades on it that will shave the ELP into small shavings, which would require much less force. One disadvantage is how dynamic this device is. It could become costly, or may not even be feasible due to all of the moving parts and material required.

**Design Three:**

![Figure 3: Section view drawing of third proposed design.](image-url)
Design three is quite different from designs one and two and uses a completely different approach to increase ELP surface area in order to increase solubility. Our first two designs had to do with breaking down frozen ELP. Design three will resolubilize viscous ELP. Viscous ELP will be poured down the end of a torsion resistant screen approximately 4cm x 6cm. Due to the viscous properties of ELP, the holes of the screen will be “filled” with ELP due to capillary action as the ELP runs down the screen. The screen will be connected to a rod, which will be connected to a high speed, high torsion motor, and the whole apparatus will look much like a fly swatter. The ELP saturated screen will then be submerged in cold PBS. The motor will then be turned on and the submerged screen will begin to rotate at a high rate of speed, producing a considerable amount of torque (amounts of torque and speed of rotation need to be determined experimentally.) When the screen begins to rotate, the force of the PBS on the ELP that is suspended in screen holes will push each small droplet of ELP out of the screen and into the solution. The screen will be rotating at a high speed, so before the ELP droplet sinks/rises and re-aggregates with other droplets that have been forced out, it will be hit again by the rotating screen, eventually becoming re-dissolved.

One advantage of this design is that it dissolves ELP in one step, no freezing required. One disadvantage would be the fact that some heat may be produced when the high speed motor is rotating the screen in the PBS solution. Heat is an antagonist to re-dissolving ELP because if the ELP is above its transition temperature, it will not re-suspend. Also, we do not know if we will be able to come up with a screen that will meet the unique specifications of this design. Lastly, the force required to push the ELP out of
the screen may be much greater than we had anticipated and we may not be able to get a motor that could supply this force.

**Future Studies**

Most of the future studies that we will be doing is analyzing and testing each one of our different designs to determine their feasibility. There are many specifications that we need to come up with still; specifications such as magnitudes of force, speed of rotation required to produce a force, and many other specifications that will determine whether or not our designs are feasible. Also, because we are dealing with a pharmaceutical drug, we will have to show our client the different design and see if we are violating any laws/rules with what we’re doing to the drug here in our different designs.
References


Appendix

Title: Device for converting ELP aggregate to soluble form

Team Members:
   Eric Lee – Team Leader
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Function:

Design a device with temperature control, salt extraction, and particle reduction capabilities to enhance the solubility of ELP (elastin-like polypeptide) aggregate in PBS (phosphate buffered saline) while minimizing product loss.

Client requirements:

- Automate the resolubilization of ELP in PBS solution
- Obtain a yield of 75-80% ELP
- Resolubilize at least 300-600 mg of ELP in 12 hrs
- Minimize the amount of PBS required to dissolve the ELP

Design requirements:

1. Physical and Operational Characteristics

   a. Performance requirements:

      The device should result in 75-80% yield of ELP and should be able to resolubilize at least 300-600 mg of ELP in 12 hrs.

   b. Safety:

      Drill operates via electrically powered motor which should not be exposed to liquid (shock hazard).
Due to the high speed of the motor and drill, one should turn unit off prior to handling (mechanical hazard from spinning components).

c. **Accuracy and Reliability:**

Resolubilization should result in a concentration of 10 - 25 μM of ELP.

d. **Life in Service:**

Ideally the device should last as long as possible (at least 12 months).

e. **Operating Environment:**

Device may be exposed to low temperature cooling media (water in the form of ice {<0°C} or dry-ice/acetone {-78°C}) as well as temperatures as high as 100 °C.

f. **Ergonomics:**

Device should be designed such that user strain is minimized.

g. **Size:**

Device should be able to fit on a laboratory bench top or in a laboratory fume hood.

h. **Weight:**

Device should be able to be lifted by one person for transportability.

i. **Materials:**

Materials that are fragile at relatively low temperatures (i.e., -78 °C) and/or high temperatures (i.e, 100 °C) should not be used.

j. **Aesthetics, Appearance, and Finish:**

There should be minimal sharp edges present on external surfaces as to minimize the chance of injury while handling.
2. Production Characteristics

   a. *Quantity*: number of units needed

      At least one unit is required for procedure.

   b. *Target Product Cost*: manufacturing costs; costs as compared to existing or like products

      Ideally under $100 for the device.