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Gene Gun Research Project

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SENIOR DESIGN PROJECT: GENE DELIVERY SYSTEM

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Introduction

Abstract

The motivation behind this project is to design or improve a cheaper gene gun that can help the world. The goal is to design a new low-cost gene delivery system that will allow Dr. Mahajan and University of Akron students conduct new research, with the aim of advancing society in many different fields. A few examples would be improving crops resistances to insects or harsh weather. This could happen by altering their genes to repulse insects or have stronger bases to have better resistance to the wind. In the medical world you could use gene therapy to help fight cancer or other diseases. The approach to this project is research, design and trial and error. The research will mostly be on how the gene delivery system drives DNA into cells using micro needles.

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Background

The goals for the gene delivery system are to have a reliable and sturdy gene delivery that can go through many trials without damage or change in how the DNA is transferred. This is important so we can get accurate and repeatable results that will allow the users of the gene gun to study how the embedded DNA affects the host. The gene gun uses pressure from compressed C02 to fire heavy metal coated with DNA at a host's cells. The metal punctures the cells, and the DNA is introduced into the cell as well. This allows the DNA to manipulate the cells. We have the opportunity to improve a design that is already built which allows us to focus on designing improvements or a better system for injection. Since we have a mostly complete gene gun, we are able to run longer trials on various design changes to collect more data before making decisions. The design goal is to have it replicate the results of the state of art gene gun as closely as possible. The current state of art gene gun is The Helios gene gun. The Helios gene gun is a convenient handheld device that uses an adjustable low-pressure helium pulse to sweep DNA, RNA, or biomaterial-coated gold microcarriers from the inner wall of a small plastic cartridge directly into target cells. The Helios cost approximately 30,000 dollars.

Biology Aspect

Definition of Chloroplast

Chloroplasts are the part of plant and algal cells that carry out photosynthesis, the process of converting light energy to energy stored in the form of sugar and other organic molecules that the plant or alga uses as food.

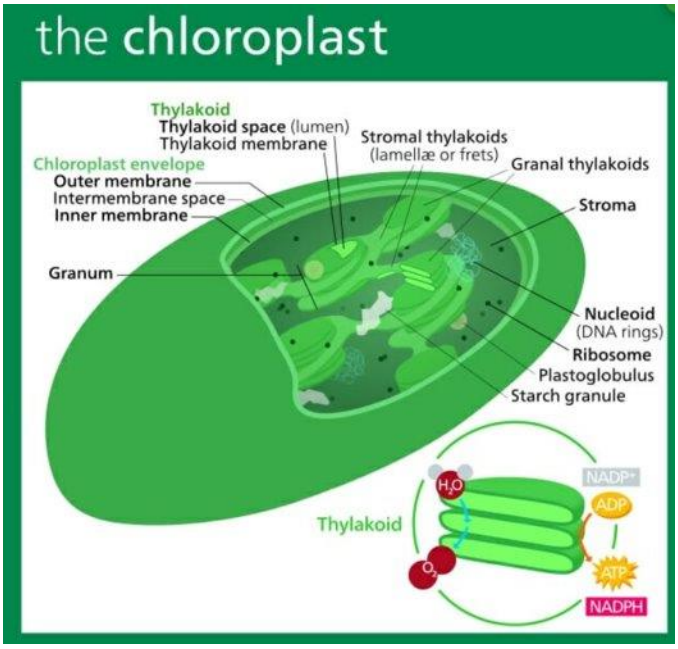


Figure 1, The shape of Chloroplast

Location of Chloroplast

The chloroplast is a cell organelle that provides energy through photosynthesis and is only located in algal and plant cells. The middle and outer membranes of the chloroplast cell are the most advantageous locations to inject the genetic fluid through, where it is mostly enriched in chloroplasts.

Like mitochondria, chloroplasts are oval-shaped and have two membranes: an outer membrane that structures the chloroplast's exterior surface and an inner membrane that lies only underneath it. A thin intermembrane space of 10-20 nanometers exists between the outer and inner membranes. The stroma is the area inside the inner membrane. The inner membranes of chloroplasts are flat, while the inner membranes of mitochondria have several folds called cristae to absorb the surface region. Instead, the stroma of chloroplasts contains a number of small disc-shaped sacs known as thylakoids.

The thylakoids in vascular plants and green algae are arranged on top of one another, and a granum is a stack of thylakoids (plural: grana). Chlorophylls and carotenoids are pigments found in the thylakoids that capture light during photosynthesis. Photosystems are structures made up of light-absorbing pigments and other molecules such as proteins. The two types of photosystems

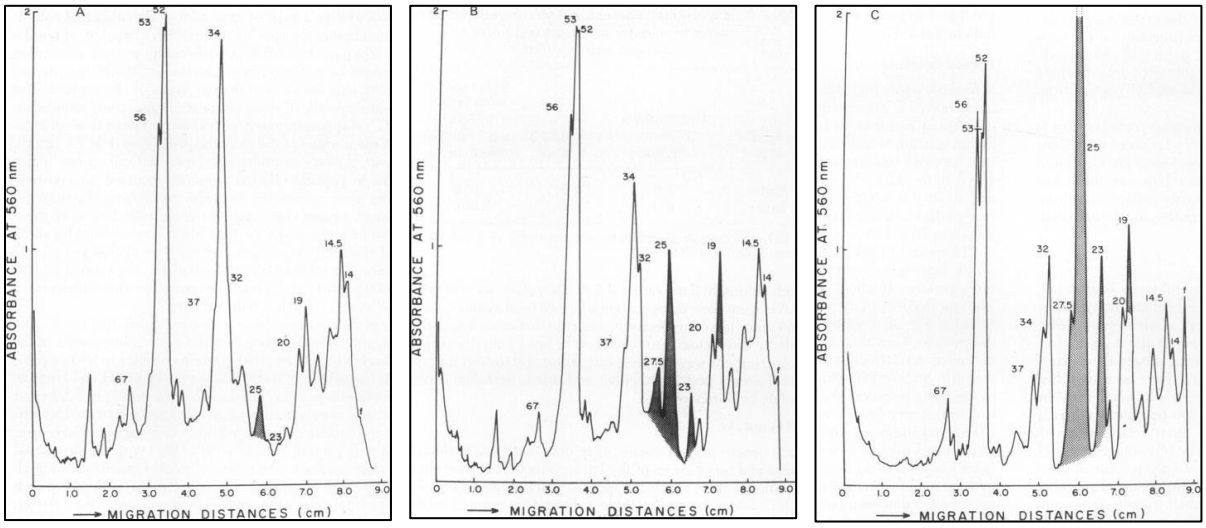


Figure (A) **Figure (B)** **Figure (C)**

Chloroplast membrane protein densitometric tracings from the inner (A), middle (B), and outer (C) leaves of a lettuce plant.

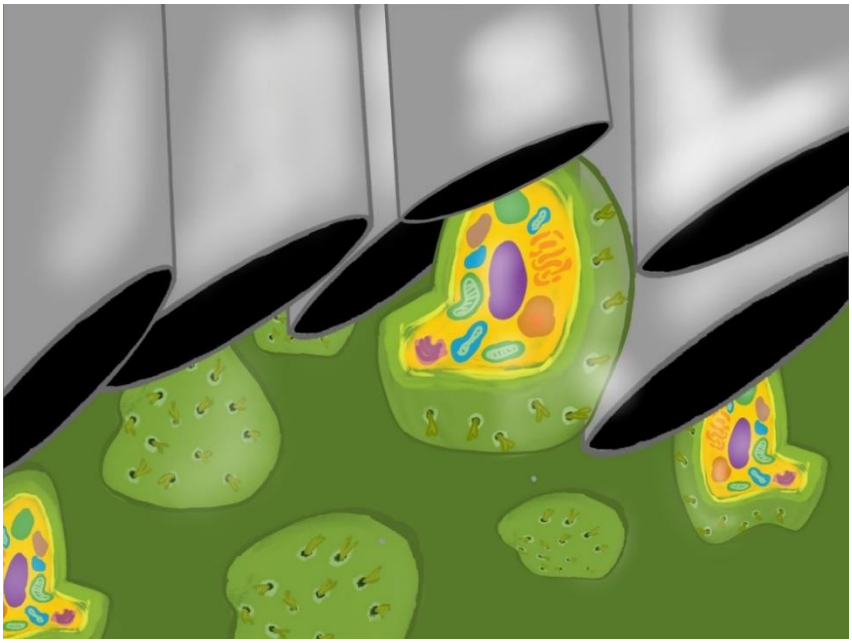


Figure 2, A microscopic drawing the shows thousands of needles injected into a lettuce leaf to reach the chloroplast

Working Principles/ Measurements

Working Principles of Syringe Gene Injector

1. To start, align the far edge of the needles at the beginning of the 1x1 lettuce plant leaf - this will put the syringe injector in the start spot (zero displacements).
2. Apply an electric voltage to the piezoelectric to change the needles' orientation. As a result of the applied voltage, the needles would be displaced onto the lettuce leaf.
3. fill the drug bottle's inlet with a liquidized drug.
4. Ascertain that the syringe plunger is fully extended. After that, manually push the syringe plunger to drive the drug into the lettuce leaf.
5. This will be repeated 10 times with 10 different distances between each repetition.

Measurements of Lettuce Leaf

By measuring 10 different 1x1 inch lettuce pieces, using a Clapper device, the follow data was collected:

Lettuce Thickness				
Sample No.	Thickness (1) (in)	Thickness (2) (in)	Thickness (3) (in)	Average of Each Sample (in)
1	0.0085	0.013	0.0105	0.01066666667
2	0.0295	0.0345	0.029	0.031
3	0.029	0.0295	0.0325	0.03033333333
4	0.0255	0.0315	0.0145	0.02383333333
5	0.04	0.015	0.039	0.03133333333
6	0.021	0.016	0.03	0.02233333333
7	0.023	0.021	0.018	0.02066666667
8	0.0255	0.0355	0.0445	0.03516666667
9	0.034	0.038	0.0335	0.03516666667
10	0.0125	0.0225	0.016	0.017
Average of each Sample			Average (in)	0.02575

Table (1)

Unit	Micrometers	Inch - Min	Inch - Max	Inch - Avg
Plant Cell Size	10 - 100	0.000393701	0.003937008	0.002165354
Cell Wall Size	0.1 - 10	3.93701E-06	0.000393701	0.000198819

Table (2)

Note: Measurement of planes cell and the size of plane cell wall. **Table No. 2**

Unit	µm	Inch	
Lettuce leaf size	654.05	0.02575	
100%	654.05	0.026	Max
90%	588.65	0.023	
80%	523.24	0.021	
70%	457.84	0.018	
60%	392.43	0.015	
50%	327.03	0.013	
40%	261.62	0.010	
30%	196.22	0.008	
20%	130.81	0.005	
10%	65.41	0.003	Min

Table (3)

Note: These data points were calculated by multiplying the average lettuce leaf thickness with a scale ranging from 10% – 100% for every 10%. Based on these calculations, we can select the needed amount of voltage to drive the piezoelectric actuator. **Table No. 3**

Unit	Inch
Needle Length	0.03
Needle Inner Diameter	0.02
Needle Outer Diameter	0.03
Number of Needles in a row	31
Total Number of Needles	961

Table (4)

Note: Measurement of Needle. **Table No. 4**

Material of Needles	Unit
Aluminum alloy	kg/m^3
Density	2712

Table (5)

Calculation	Unit	
No.961 - Needles Wight	0.000172	kg/m
Needles Volume	0.000009	inch ^3

Table (6)

$$W = \rho * \pi \frac{\left(d_o^2 - d_i^2\right)}{4}$$

Equation (1)

W = weight of empty pipe per unit length (kg/m, lb/in)

ρ = density of pipe material (kg/m^3, lb/in^3)

d_o = outside diameter (m, in)

d_i = inside diameter (m, in)

$$V = \pi * r^2 * l$$

Equation (2)

V = Volume

r = Inner radius (Inch)

l = length (inch)

Results

Probability of Hitting Chloroplast

The probability of a single needle hitting the chloroplast of a plant cells is somewhere between 28% and 48%, depending on the species and size on the plant. This is due to the general size of the chloroplast ranging in about 5 to 7 micrometers long and can number from 1 to 100 in a single cell. The size of the cell also ranges dependent on the species. the calculation on these were done by averaging the size of the chloroplast multiplied to the average number of chloroplasts in a cell, then divided by the total size of the plant cell. Our high-performance

change was taken by having the larger chloroplast in the Higher count to the largest plant cell size.

Design

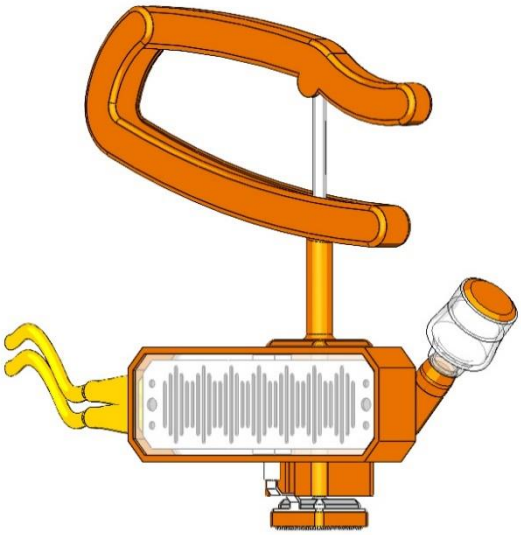
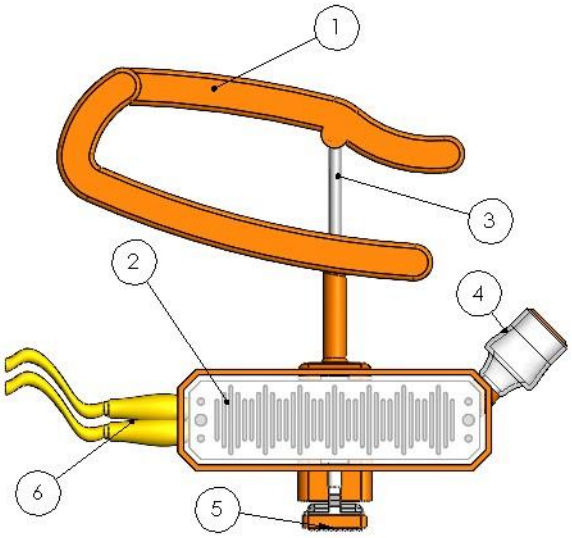


Figure 3, Syringe Gene Injector

Simple Design Brief

The Syringe Gene Injector is a device that allows a large number of living cells to be injected with a genetic liquid drug material. Using 961, micro needles to drive the fluid flow into a lettuce plant leaf. This Mechanical approach will allow a large amount of genetic fluid to transfer into the plant cells while leaving the cells healthy enough to function normally. The

figure below will provide some information about Syringe Gene Injector parts and quantities of each part.



ITEM NO.	PART NUMBER	QTY.
1	Syringe Gene Injector	1
2	Pizoelectic Actuator	1
3	Syringe Plunger	1
4	Drug Inlet Bottle	1
5	Needles	961
6	Electric Source	1

Figure 4, Parts of device & quantities

Piezoelectric

A major problem faced during this project was how to accurately position the microneedles inside the lettuce leaf. A high degree of precision would be needed, so we decided to use a piezoelectric actuator. The actuator will move the microneedles into the lettuce and then the liquid can be forced through the needles by hand. Piezoelectric actuators are capable of nanometer precision while also generating high amounts of force making them ideal for this design. Other benefits of using piezoelectric actuators include fast response times and a long cycle life. Operating a piezoelectric actuator requires both a signal generator and a voltage amplifier which will be discussed later.

Choosing the actuator was done using the data we gathered from measuring lettuce leaves, our data found the largest travel we would need is around 650 μm. To ensure our design could exceed this requirement an actuator with a travel range of 1000 μm would be chosen. The P-602.8S0 Piezo Move High-Stiffness Linear Piezo Actuator fits this requirement and was chosen for our design. The actuator also can generate 100N of force which is more than enough to position the microneedle head. This actuator is a flexure-based actuator meaning, it is composed of several layer of piezo ceramic materials that bend which creates displacement vertical to the bend.

Choosing the amplifier and signal generator is dependent on several specifications of the chosen actuator. Several important factors were the voltage range and peak current requirement of the actuator. Fortunately, the manufacture of the actuator recommends several controllers that fit these requirements. The amplifier we chose is the E-610-S0 Piezo Amplifier / Servo Controller. This serves both the function of voltage amplifier and a servo controller eliminating the need for two separate pieces of equipment. The system will operate on a closed loop control system allowing for greater resolution of movement. A closed loop control system will take advantage of the built-in strain gauge sensors on the P-602.8S0.

Positioning the microneedle head using a piezoelectric actuator allows for different voltages to correlate between different positions of the microneedle head. The equation showing this relation is given below taken from the producer of the actuator's website. The actuator we are using is an all-ceramic flexure actuator which is why this equation was chosen.

Equation (1) $\Delta L_{bend} = \frac{3}{8} n d \frac{l_f^2}{h_p^2} V$

$L_{bend} = Bending\ Displacement$

$n = Number\ of\ Stacked\ Cermaic\ Layers$

$d = Transverse\ Pezoelectric\ Large - Signal\ Deformation\ Coefficient\ [m/V]$

$l_f = Free\ Bender\ Length$

$h_p = Height\ of\ Piezoelectric\ Element$

Several of the variables in this equation are not known to us, as they are inherent properties of the piezoceramic material, and the manufacturer does not list this information on their website. However, variables d, n, lf^ , hp^ are constant and will be the same at any voltage or displacement of the actuator. Next, we can set these variables all equal to a constant called x. This allows us to rewrite Equation (1) as Equation (2) shown below.

Equation (2) $\Delta L_{bend} = \frac{3}{8} xV$

Lastly, we know the actuator will produce its largest displacement at the largest voltage within its operating range. From the data sheet of the actuator, we know max ΔL_bend=1000 μm and max V is 120 V. Putting these values into Equation (2) gives x=2.22e-5. This value can now be used in Equation (2) to calculate the voltage needed to reach a desired displacement. Results of using this formula from a range of 0 to 120 V are shown in Figure (1)

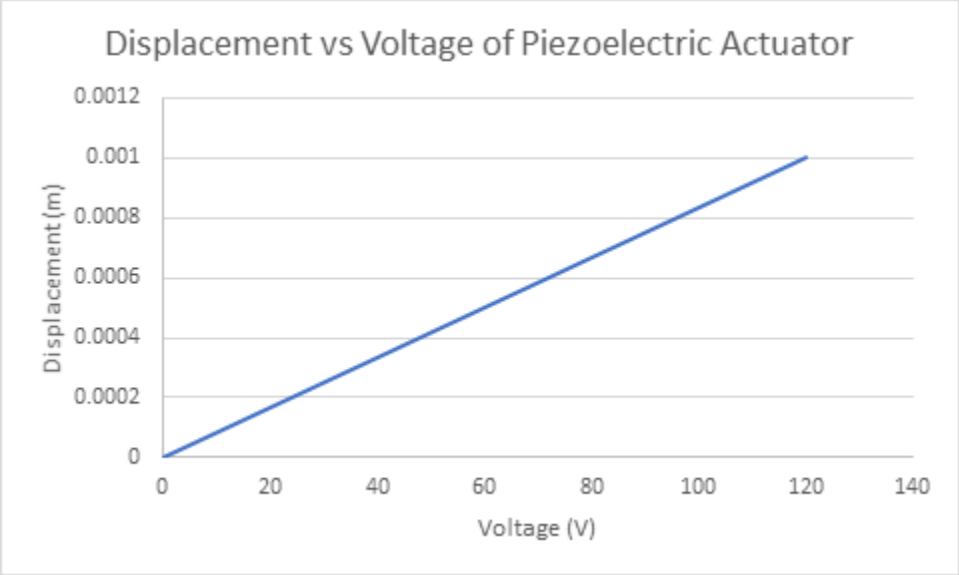


Figure (1) Displacement vs Voltage of Piezoelectric Actuator

Figure 5, The P-602.8S0 Piezoelectric Actuator

3D printing

3D printing or additive manufacturing is a process of making three dimensional solid objects from a digital file. We are using 3D printing to print our design for this project because it is cheap and with high quality by using Solid-Work. We are printing out the parts by using PLA (Polylactide) material. PLA material is highly recommended for 3D printer as it is easy to print with excellent surface finish, wide range of color options, high stiffness, biodegradable and environment friendly.

We are also going to use 3D printing to print the microneedles parts by using aluminum material because of the size of the needles are going to be super small. microneedles were observed to be shrunk and bent. So mechanically, microneedles should be strong. However, some of the polymers used to make microneedles don't have enough power to make arrays. Furthermore, for the needles as the famous facility has created a method for 3D printing high-strength aluminum. That’s why we are using aluminum for the needles. Furthermore, we are also printing the plunger and after we are going to print it, we will use a rubber material to go in the top of it. The benefit of the rubber Usually the needle pushes the rubber aside and it “self-heals.” The rubber is made from two types of materials: bromobutyl or chlorobutyl elastomer. Also, it is going to help us with the stability and will not be lose.

Discussion/ Conclusion

Based on all research, we found that the best places to transform genes through lettuce are the outer & middle layers of lettuce cells. And that is because the outer and middle layers of cells contain the majority of Chloroplast (Chlorophyll) in lettuce. Our target and goal are to design a needle delivery system that transfers liquid drugs to lettuce leaf by injecting the drugs into the outer & middle layers of chloroplast in a lettuce cell. Our final model is the “Syringe Gene Injector” and allows a large number of living cells to be injected with a genetic liquid drug material. We will use 961 micro needles to inject the liquid. The needles are driven by a piezoelectric actuator for nanometer precision with high force. The probability of a single needle hitting the chloroplast of a plant cells is somewhere between 28% and 48%. In conclusion, the gene delivery system developed is an innovative design that still needs to be physically created and tested.

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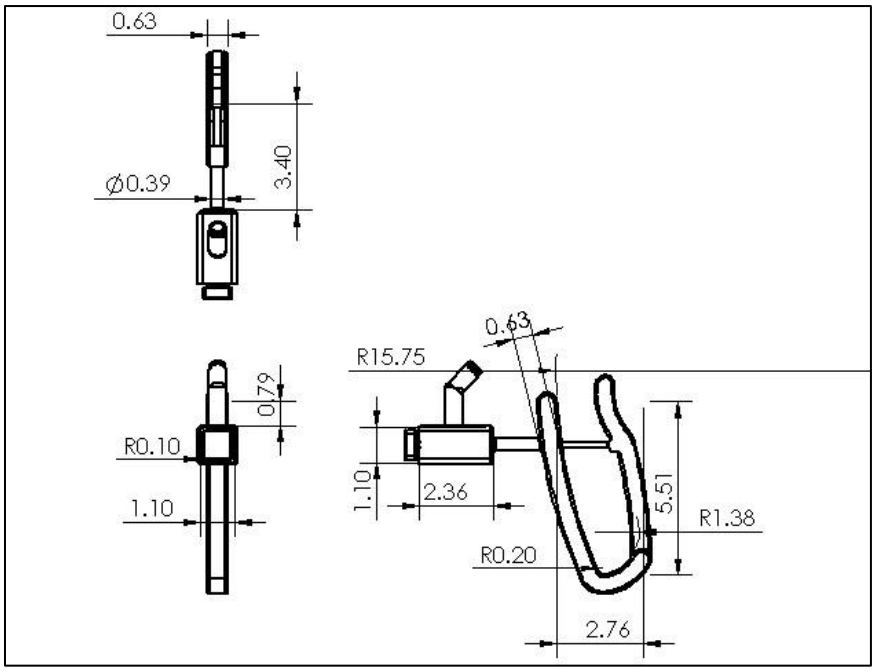
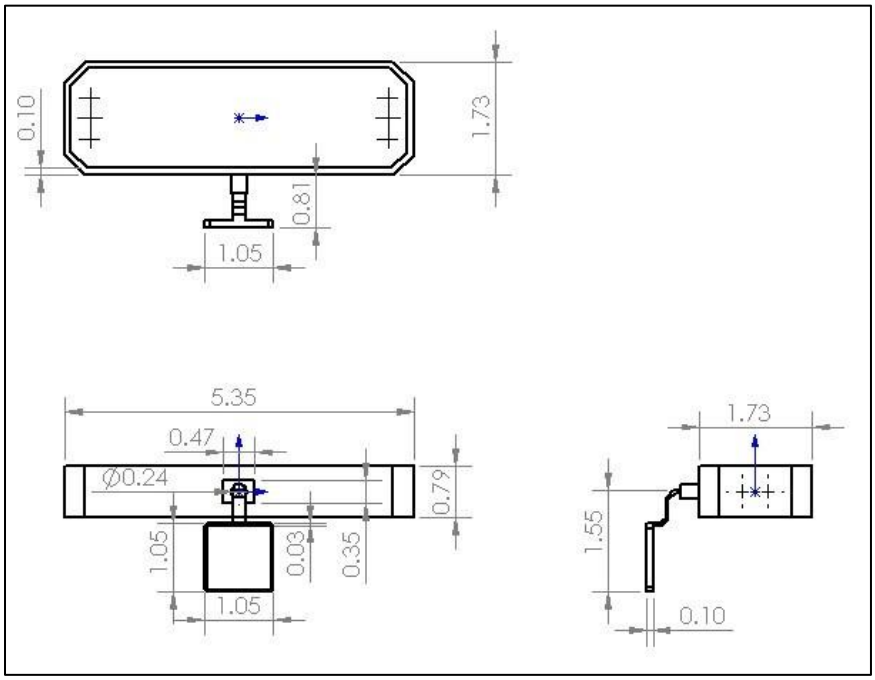
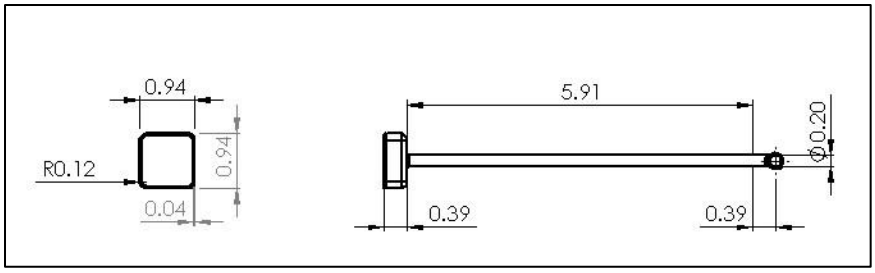
Appendix 1



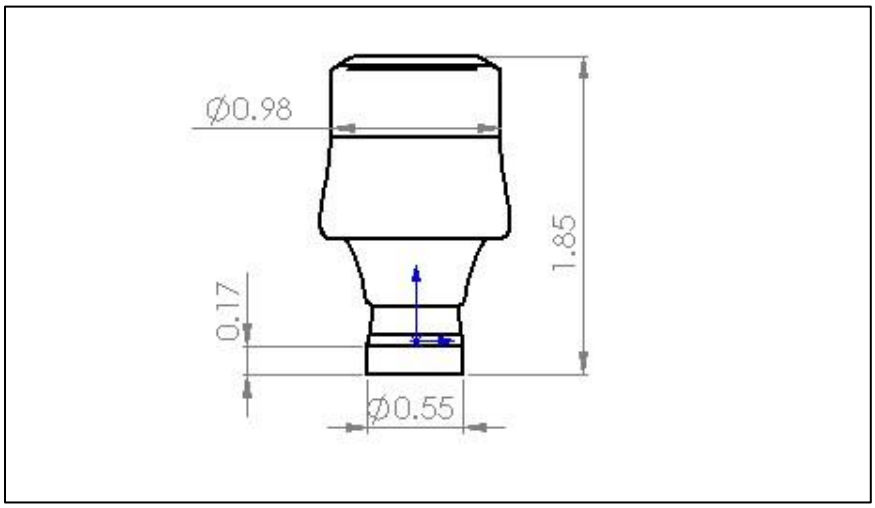
Syringe Gene Injector Frame



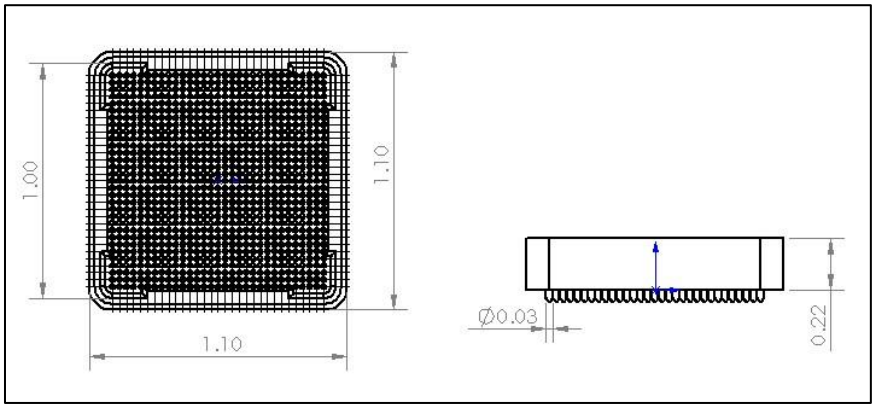
Piezoelectric Actuator



Syringe Plunger

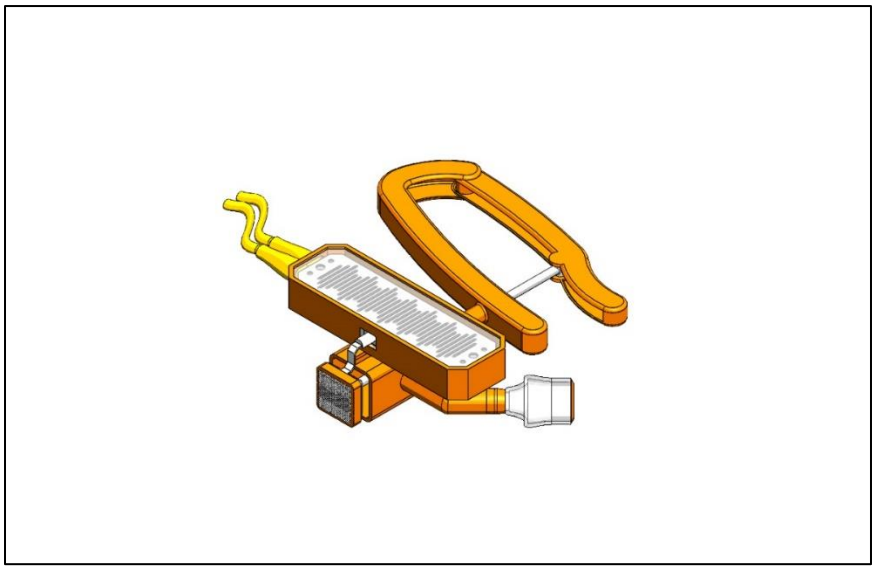


Drug Inlet Bottle

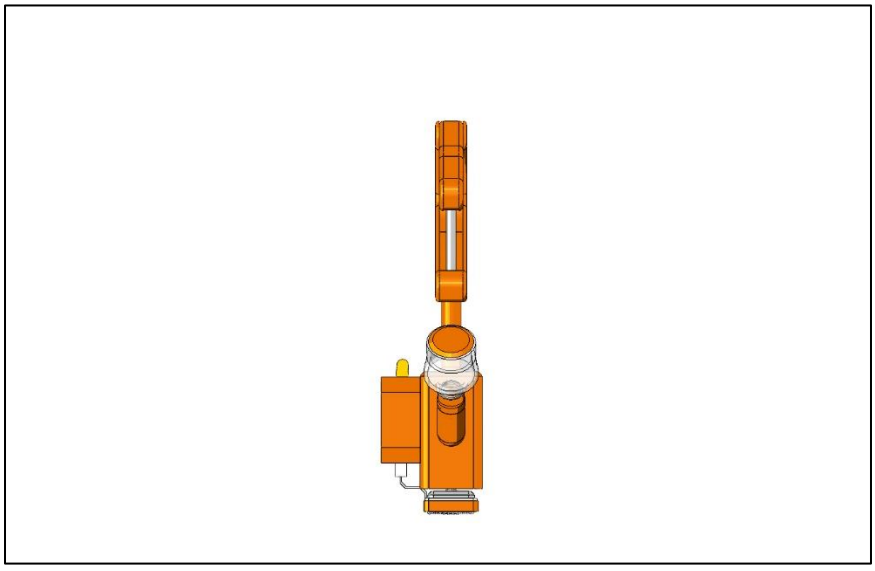


Needles

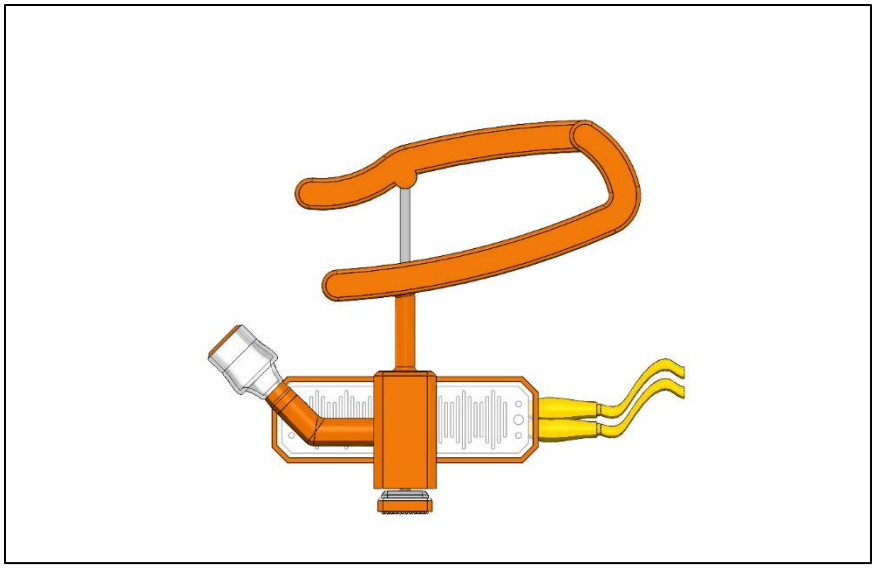
Appendix 2



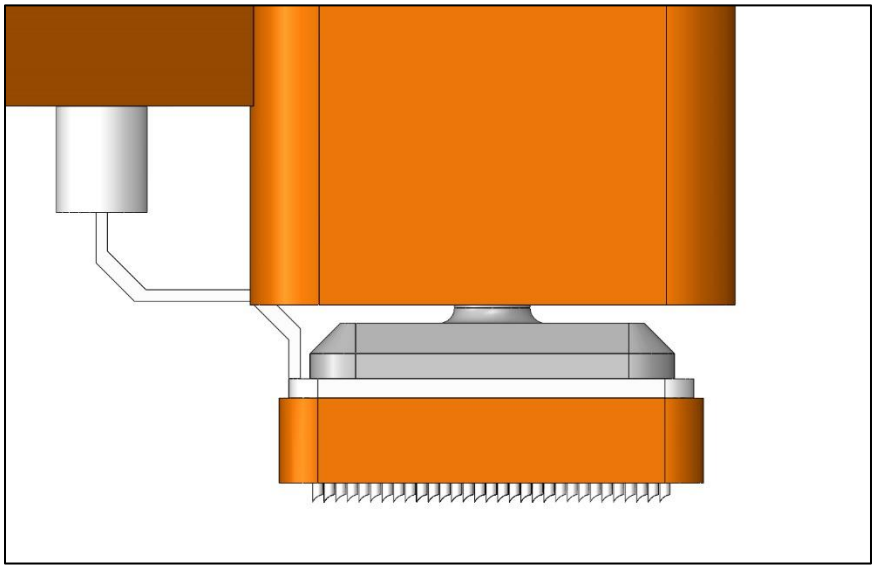
Isometric View of Syringe Gene Injector



Front View of Syringe Gene Injector



Side View of Syringe Gene Injector



Zoomed View of 961 Needles