PLGA Scaffold Device

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Statement of Disclaimer

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1.0 Executive Summary

PLGA is used to create three dimensional porous scaffolds that play a crucial role in osteoblast proliferation. This technique can be helpful for cell transplantation and other bone research applications [5]. The properties of the scaffolds may cause them to float in the surrounding media, exposing the top surface to air where cells can't proliferate effectively. The purpose of the PLGA Scaffold Device is to submerge the scaffolds in the cell media to promote cell growth and proliferation. While achieving this main goal, the device also had satisfy several customer requirements such as being biocompatible, sterilizable and able to survive incubation. A final design was chosen to replace the lid of the well plate to submerge the scaffolds. Cell proliferation, autoclave and incubation tests were performed to ensure previously customer requirements were met. This document overviews the development of a cost effective device that will work in combination with a well plate to keep the scaffolds submerged.

2.0 Introduction and Background

The purpose of the PLGA Scaffold Project is to design a device that keeps PLGA scaffolds completely submerged in a cell nutrient medium for up to three weeks. The submersion of the scaffold will lead to greater cell proliferation and growth on the top surface of the scaffold. This device is being designed for Christopher Heylman bone tissue engineering research efforts.

This document will overview existing products and patents similar to our device and why there is a need for such devices. We will also discuss our customer requirements, translated into engineering specifications, and corresponding relevant standards and codes. We will lay out initial plans for the design process and how we want to manage the project over the next quarter.

Current Products:

There are several current products on the market that address flotation of scaffolds in the cell medium. Thermo Fisher Scientific Cell Culture Inserts for Skin Tissue Culture includes a well plate with polycarbonate membrane inserts. There are 3 different height ranges for the inserts. A case of twenty-four 6-well plates with inserts included costs $181.

Master bond has a MB250NT glue which is an cyanoacrylate that is non-toxic and adheres with ISO-10993. The glue can be used to adhere scaffolds to the well plate. MB250NT glue adheres to ISO-10993 standards, cures rapidly and is resistant to gamma sterilization. A two ounce bottle of MB250NT glue costs $40.

In several experiments, researchers have placed stainless steel rings on top of scaffolds to keep the submerged. In one study, the stainless steel ring was made by the University of Nottingham[3]. The estimated cost of the stainless steel ring is $50.

Falcon Cell Culture Inserts have a porous membrane on the bottom of the insert. The membrane has two pore size options and two pore density options. The inserts are low protein binding,sterilized by gamma irradiation and cost $248 for forty-eight cell culture inserts.

Cell Crown24NX inserts are made from a polycarbonate material, available for 12 and 24 well plates, the height of th inserts can be adjusted, they can be delivered gamma-irradiated or

non-sterile. The main difference in this project is the insert, sandwiches the material and can completely submerge the sample in the cell medium.

The products above were difficult to find online, which implies they are not widely used. In numerous research papers the scientists, would design their own device to keep the scaffold submerged. This also implies that an adequate product has not yet been designed that allows for ideal cell proliferation and keeps the scaffold completely submerged. The previous products could help us brainstorm different design ideas or expand and improve the designs already on the market.

Patents:

***Table 1*:** Patent Information on Scaffold Stabilization Methods and Devices

Patent	**Characteristics**
US5578492A Cell Culture Insert[7]	● Cell supporting membrane separation device ● Support mechanism for holding scaffold suspended in medium ● Break-away mechanism to separate from the support mechanism
US6468788B1 Method and Device for Accommodating a Cell Culture[10]	● Vessel has base, walls and lid enclosing scaffold in cell medium ● Evacuation opening on lid for excess liquid and displaced air
CN106367347A Biological Support Material Fixed Mount[2]	● Biological scaffold bracket including a fixed portion, and a controllable vertical bracket portion ● A vertical portion connecting the fixed bracket and control unit ● A controllable portion rotatably connected on the upright support, ● Grooves along the controllable portion help hold each scaffold in the slot on the controlled portion
CN103396935A Biological Scaffold Material Fixing Rack[11]	● Vertical support section again cell wall ● Two ring arm encompassing scaffold ● Adjustable ring size and height ● Spring vertical bracket hung above medium to hold vertical support in place
WO2017141531A1	● Scaffold holding plate ● Movable component that holds the

Method for Seeding Cells to Scaffold Material[12]	syringe with the cell medium ● Needle has multiple discharge points ● Syringe is movable forward and backwards

Table 1 shows five patents for securing biological scaffolds and ensuring they do not float. Each patent has a different approach to achieving this goal. The Method and Device for Accommodating of a Cell Culture patent is basically a cage that contains the scaffold and keeps it submerged in the cell medium. The Method for Seeding Cells to Scaffold Material patent is seeding cells with a syringe therefore eliminating the need for submersion in a cell medium. The Cell Culture Insert is similar to the Cell Crown24NX inserts described in the previous section of Current Products.

Technical References:

The purpose of tissue engineering is to re-establish or mimic the function and output of a tissue or organ. The basis of tissue engineering involves cells and a scaffold. In almost all cases cells cannot simply be injected into the damaged tissue or organ to restore function. Scaffolds are used to support cell growth, proliferation just as the extracellular matrix does. A scaffold placed in the body at the point of regeneration can also serve to protect the site of action from attacking cells in the body[6].

There are around 1 million individuals with a skeletal defect requiring a bone graft every year. Tissue engineering efforts focusing on bone are trying to eliminate the need for autologous grafts and allografts. Autologous grafts are limited due to donor site morbidity and the limited amount of bone that can be excised[9].

Scaffolds have to meet certain requirements to effectively allow for cell growth and proliferation. The pore size of scaffolds is important because it affects the mechanical strength of the scaffold the pores also need to adequately sized for nutrients, waste and gases to move through the scaffold[9]. The pores also need to be interconnected to form a network that allows for the cells to proliferate throughout the whole scaffold. The rate of degradation of the scaffold is also important and dependent on the tissue being regenerated. The degradation rate of a scaffold for the skeletal system would be slow to allow for the bone to heal and grow. If a scaffold remains longer than needed, it could negatively affect the cell growth and function of the native tissue[6].

The gas-foaming method is used to create PLGA scaffolds. For this method, sodium bicarbonate,a foaming agent, is added to the polymer phase of PLGA. This mix is compressed into a disk and placed in a compression chamber with CO_2. The gas phase rises to the surface of the structure, while the liquid phase sinks to the bottom. Once the gas has completely left the polymer, a porous structure remains due to the gas particles.The top of the scaffold tends to be more porous due to the gas diffusing up and the bottom of the scaffold tends to be less dense due

to the liquid moving down due to density differences[4]. The sodium bicarbonate is then leached out of the scaffold to ensure the outer layer of the scaffold is porous and the pores are interconnected [1]. The foam is then stabilized with the addition of a surfactant. The surfactant also prevents liquid from draining from the scaffold [4].

PLGA is widely used in tissue engineering of bone due to its controllable degradation rate and biocompatibility. Both lactic acid and glycolic acid are naturally occurring. Once the scaffold is degraded, both acids are removed from the body through natural pathways [5].PLGA is a copolymer of poly lactic and co-glycolic acid formed through a condensation reaction. PLGA is widely used due to its controllable degradation rate. This is determined by the ratio of glycolic acid to lactic acid. Lactic acid is more hydrophobic which leads to fewer ester linkage breaks and a slower degradation rate. A limitation of PLGA is that it does not perform very well in load bearing situations due to its significantly lower Young's Modulus than bone [8].

Knowledge of the purpose of tissue engineering scaffolds, the procedure to make them and the ideal properties of the scaffold will give our group a more comprehensive outlook and perspective on PLGA scaffolds. Porosity, pore size and degradation are important for scaffolds to proliferate cells and grow cells. For our design, we now know our device should not adversely affect these properties. Other information from these technical papers will help us later on in the design stages when we have a more definitive direction.

Project Specific Information:

During the first week, the PLGA Scaffold group met with Dr. Heylman and discussed the details of his bone research lab. We learned the PLGA scaffolds are around 5 mm in height and that the PLGA scaffold procedure includes incubating the scaffolds for a maximum of three weeks. After hearing that the device would be incubated, material selection became a high priority. Designs that were found in research articles were discussed with Dr. Heylman and he said his lab had considered a cage design for the device to keep the scaffolds submerged. Information specifically regarding Dr. Helman's PLGA Scaffolds will help us create a design that is tailored to his lab and procedure.

Standards, Codes and Regulations:

ISO 10993
ISO 13485
MSDS
Approved Biomaterials List
Standards put out by CDER, CBER and CDRH
GLP and GMP

3.0 Customer Requirements and Design Specifications

3.1 IFU

A method to ensure the entire scaffold is submerged and/or exposed to medium. This method will be used for tissue engineering applications to ensure scaffold submersion in media to promote cell growth throughout the scaffold.

3.2 Product Design Specifications

Table 2: PDS matrix for our PLGA scaffold device.

Customer Requirement	**Engineering Metric**	**Specification**	**Rationale**
Must be sterile and portable enough to be placed in humidified cell culture incubator	Must withstand 5% CO2 and 37°C incubation environment	No temperature warping at 37°C in humidified environment	Must survive temperature similar to human body to allow accurate osteoblast growth.
Must be cost effective	Low Cost	Under $5	The allocated budget is set from the class requirements
Must be disposable or, if reusable, must be sterilizable	Approved biomaterial, can be autoclaved	No change in dimensions after autoclave	Many scaffolds will be used. Reused scaffold supports must be sterile to allow proper cell growth.
Must survive throughout entire incubation	No degradation anywhere from 7 days - 3 weeks	Keep X% of UTS after 3 weeks of media contact	Degraded scaffold will alter cell growth patterns midway through incubation.
Must allow for cell proliferation on top layers of scaffold	Small contact area with scaffold	Contact area less than 5 mm2 per scaffold	To promote maximum cell growth in the scaffold.
Must be able to attach to well plate	Similar length and height of well plate	Fit around well plate with 1.5 mm or less in clearance	To stabilize the device on the well plate to avoid movement of the scaffolds.

3.3 House of Quality

Table 3: HoQ rooms 1, 2, & 4.

		Engineering Specifications					
Improvement Direction		n/a	↑	↓	↓	↓	↑
Units		n/a	MPa	$	mm^2	in or mm	lb
Customer requirement	**Importance factor**	**Biocompatible material**	**Material strength**	**Low cost**	**Small contact area**	**Dimensions within 1" of well plate**	**Weight**
Sterilizable or reusable	**5**	9	3				
Cost effective	**3**	3		9			9
Survive incubation	**5**	3	9				
Allow top surface proliferation	**4**				9	1	
Attach to well plate	**4**					9	1
Raw Score	**263**	69	60	27	36	40	31
Relative Weight %	**100**	26.2	22.8	10.3	13.7	15.2	11.8
Rank Order	-	1	2	6	4	3	5

Table 4: HoQ room 3 screenshot from excel.

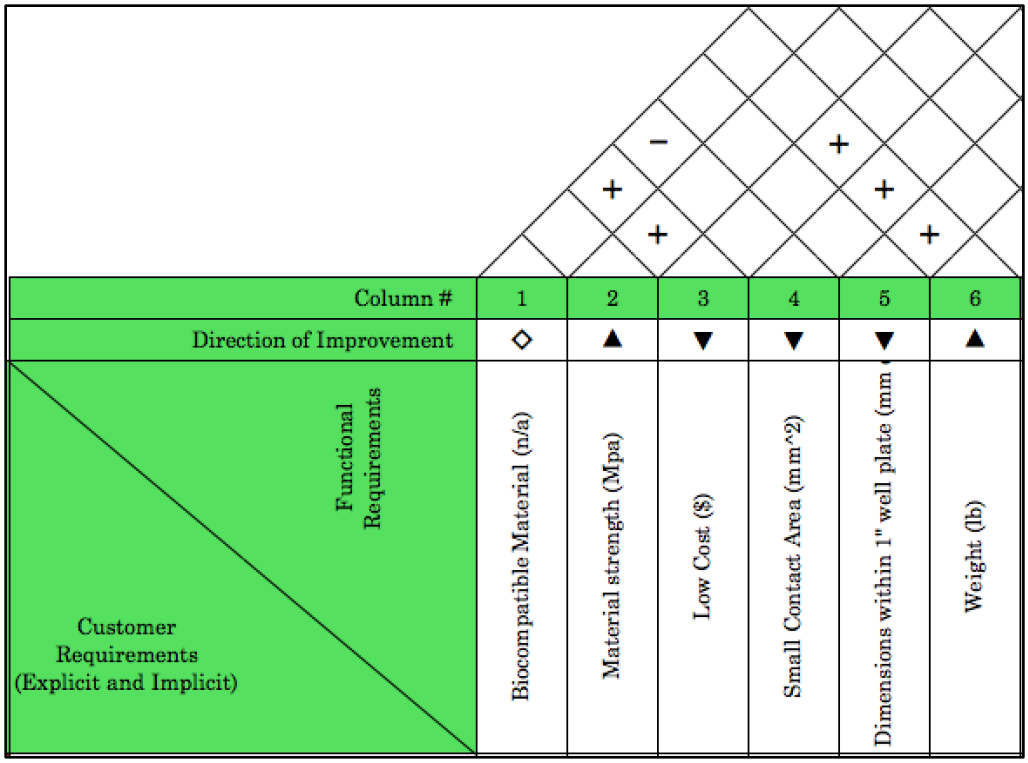


Table 5: HoQ room 6.

COMPETITOR RANKINGS 1-Poor, 3-OK, 5-Excellent			
CR	CellCrown 24 NX Well Inserts	Master Bond Cyanoacrylate	Our Device
Sterilizable or Reusable	1	2	4
Attach to Well Plate	5	4	5
Allow top surface for Proliferation	3	3	4
Biocompatibility	5	5	5
Cost Effective	3	3	3

Survive Incubation	3	5	5

4.0 Stage Gate Process

A PERT chart was created at the beginning of this quarter to determine what tasks needed to be completed and when to finish the project on time. The critical path is mainly determined by class presentations. Our PERT chart ends with two deliverables: the Final Design Presentation and the final report. The next tasks on our critical path are the Manufacturing Plan, Material Selection, and Design Freeze Presentation. To complete these tasks, dimensions for our device, details of the design and clear, chronological manufacturing instructions will need to be completed. The PERT chart can be referenced below in Appendix B.

4.1 Concept Review

For our project three main designs were considered. The first design was an external device with a large base and prongs. The prongs would have tips that are in contact with the scaffolds and submerge them in the cell medium.The top of the device would serve as the well lid and the legs of the device would attach to a base below the well plate. This concept allows contents of all wells to be submerged simultaneously.

The second design was a semipermeable membrane spherical shape with a semipermeable lid. A handle would attach to the semipermeable membrane and hook onto the well. The semipermeable membrane would allow for the scaffold to have contact with the medium and remain submerged. This design would require a device for each individual well. This concept requires tedious setup but allows all scaffolds to be submerged and removed simultaneously.

The third design was a bioreactor. The bioreactor would be enclosed in a container and have one flow chamber. The flow would be in the direction of gravity and would flow through a funnel shape that would be larger at the top than the bottom. The fluid flow would serve to keep the scaffold submerged in the cell medium without constant media change. Each scaffold would have to have its own cell in the bioreactor. This design is significantly more expensive but negates the need for a well plate or constant media change.

PUGH charts were made comparing each design to a current product on the market, Cell Crown24NX inserts, and comparing each design to each other. The PUGH charts can be referenced below in Appendix E. The net number of positives for each design was assessed and a front runner concept of the external device with a large base and prongs was selected.

4.2 Design Freeze

The first design was chosen, with reference to Concept Review, and designed in 3D modeling software, Solidworks. The design was demonstrated as replacement to the lid to work

in conjunction with the already existing well plate. The design was made to physically push the scaffolds into the media, forcing the entire scaffold to rest in the media. The prongs were varied in size and amount for variation in determining the highest efficacy for cell growth.

4.3 Design Review

An effective base thickness was needed to correspond with the 3D printers effects on the material. The prongs were increased by .5 mm in diameter for a higher efficacy in printing. Lastly, the optimal number of prongs and size was determined to be n=3 prongs and 1.5mm diameter, *7.0 DOE*.

5.0 Description of Final Prototype Design

5.1 Overview

The device will be composed of all Nylon PA 6 and replace the lid of the well plate. It will be designed to be the same basic size as a normal well plate lid. Prongs attached to the underside of the lid will project into each well and hold down the scaffolds. Each 'prong' consists of solid cylinder base projecting from the lid with smaller cylinders projecting off the base cylinder. These small cylinders will be in contact with the scaffold. It will be intended for a 48-well plate and have 48 prongs.

5.2 Design Justification

Tolerances were given from the Ultimaker printer manufacturer website. All dimensions in the drawing are millimeters. The device will be printed upside down so that the prongs will project from the lid upwards. SolidWorks drawings can be seen in appendix C.

5.3 Analysis

Using original lid design measurements will ensure a secure fit over the well plate and maintain a small size. The device will be one solid piece to increase its durability. Initial prototypes proved that minimum prong diameter must be at least 1.5 mm for proper printing. The smallest possible diameter should be used to minimize surface contact with the scaffold.

5.4 Cost Breakdown

Table 6: Bill of materials.

Product	Distributor	Cost	Unit	Amount	Details	Product Number	For
Nylon 6	3D Universe	40.49	each	1	750 g spool of 2.85 mm filament	KODAK_NY63NON	Manufacturing
Garolite sheet	McMaster Carr	6.82	each	1	12" x 12" 1/32" thick	9910T11	Manufacturing
Ultimaker 3 3D Printer	3D Universe	3495.00	each	1	Complete 3D Printer	UM3	Manufacturing

We have approved access to the 3D printer, the spectrophotometer, and the autoclave. We will not need to spend money on manufacturing or testing besides the materials. Everything thing can be done at Cal Poly with help from students and faculty.

5.5 Safety Considerations

The device will contain small detailed design that can lead to sharp edges or prongs breaking off. The sharp edges are smoothed after the autoclave process, so the device should be handled carefully or with gloves before the sterilization. Prongs should never be pushed or touched with fingers to prevent damage to the smaller parts.

6.0 Prototype Development

6.1 Model Analyses

Manufacturing of our device was done in a lab containing a Ultimaker 3 3D printer. No outside manufacturing services were needed. Our parts were machined printed to size. Our device contains many small parts which could be difficult to manufacture. Also, tolerances of the prong diameters and spacing are important to ensure each prong lines up over a specific well.

The tip design of our prongs were further developed. We wanted to minimize media displacement and scaffold surface contact. Therefore we altered the tip where it contacts the scaffold, for efficacy of 3D print and connection with the scaffold.

6.2 Evolution of Prototypes

The original prototype contained a large variety of prong types and prong lengths. The first prototype consisted of many manipulations that weren’t consistent with 3D CAD design.

The second prototype increased the base thickness for printing efficacy. The purpose was due to warping of the base portion. The diameters of the prongs were all increased by .5 mm in diameter to increase the efficacy of print. Therefore all prongs had either a 1.5 mm or 2 mm diameter.

The final functional prototype contained a range of diameters and amount of prongs in order to determine the best configuration for cell growth.

6.3 Manufacturing Process

The manufacturing process consisted of 3D printing the CAD design with Nylon PA 6 on an Utilmaker 3 3D printer.

MPI

1.) Purchase 2.85 mm Nylon PA 6
2.) Save part file "Scaffold_Lid.sldprt" as an .stl file
3.) Save SW file on USB/SD card or send to printer operator
4.) Load Nylon PA 6 into the Ultimaker 3
    -On the printer select 'material' -> 'change'
    -Wait while nozzle heats up, filament will start retracting automatically
    -Once printer says 'Insert New Material' place spool so it spins CW as it is used
    -Press 'continue'
5.) Place Garolite Sheet on glass bed
6.) Program heating bed and printer to desired printer settings
    -Print temp 240-280C
    -Print bed 90-120C, not glass
    -Cooling fans: off
    -Slow speed (<40 mm/s) for first ten layers to avoid warping
7.) Print for specified time according to Ultimaker 3 display

Table 7: Design history record.

Step #	Deviation from MPI	Date Performed	Signature
1	N/A	1/15/19	Bryce
2-3	N/A	1/29/19	Tyler
4	Printed on masking tape, not garolite*	1/30/19	Tyler
5-7	N/A	1/30/19	Tyler
5-7	N/A	2/2/19	Tyler
5-7	N/A	2/7/19	Tyler
5-7	N/A	2/28/19	Tyler
5-7	N/A	3/17/19	Tyler

*The Garolite sheet was not large enough to cover the entire print bed, layers of masking tape worked well.

6.4 Divergence Between Final Design and Final Functional Prototype

The final design and the final functional prototype will be made using the exact same manufacturing process. The only difference is that the final design will have uniform prongs that are all exactly the same. The final functional prototype has multiple prong types that differ in size and the number of contact points, which will be evaluated during the final cell proliferation testing. The final design will be made using the single prong design that allows the most cell growth.

7.0 IQ/OQ

7.1 DOE

Engineering Metric	Specification	Test Method	Test Apparatus Location	Apparatus Experience / Training	Sample Size	Power
Must endure incubation environment, 37 C 5% CO2	Less than 5% of adhesion strength loss	Epoxy adhesion test	192-329	Flexural tester set up/run program (420)	n=2	.8
Low cost	Under Allocated Budget	-	-	-	-	-
Must be approved biomaterial, sterilizable or disposable	Biocompatible and non-toxic FDA approved material	MSDS ISO 10993	-	N/A	n=2	.8
Must allow cell proliferation of top surface of scaffold	Maximum surface cell coverage	Stain for osteoblasts from section of top of scaffold	128-329	Slide preparation and staining (420)	n=2	.8
Must be able to attach to cell plate	Within 1 inch of dimensions of well plate	Dimensions of cell plate measured	192 PLGA Scaffold Lab	Ruler experience	n=2	.8

7.2 Verification and Validation

Cell proliferation testing

Two cell proliferation tests will be performed. One test will be with performed using NIH-3T3 fibroblasts without scaffolds or our device. The other test will use our device and have cells seeding on scaffolds. Both tests will use a 48 well plate, liquid nutrient media, and proliferation will be quantified using a CCK-8 assay from Sigma Aldrich.

<u>NIH-3T3 Testing</u>

Equipment: 48 well plate, NIH-3T3 cells, nutrient media, CCK-8 assay, micropipette, gloves, 15 mL conicals, DI water

Location and time: 192-328 (plating and incubation) and 33-394 (absorbance measurement) on Friday 3/1/19 from 8-11 a.m. Cells were plated the previous wednesday around 12 noon.

Procedure:

1.) Obtain all equipment and sterilize if necessary.

2.) Plate NIH cells onto the plate according to the following:

-Six wells are used for each of the five concentrations, totaling 30 wells.

-Concentrations used are: 6250, 12500, 25000, 50000, & 100000 cells per well.

3.) Once plated, pipette 0.50 mL nutrient media into each well with NIH cells.

4.) Cover well plate with well plate lid and place in incubator at 37°C, 5% CO_2 for 44 hours.

5.) Remove plate from incubator, remove lid, and add 30 microliter of CCK-8 cellular assay to each well with cells and media.

6.) Fill a 15 mL conical with 3.0 mL DI water and 30 mL CCK to act as a control.

7.) Place well plate and conical with DI water in incubator for 3.5 hours. Remove well plate.

8.) For wells of the same concentration, pool each of the six wells into a 15 mL conical using a pipette. (fig.1) You should now have five 15 mL conicals each with 3.0 mL of sample, as well as the one with water from step 6.

9.) Cover the conicals with tinfoil and take them to the spectrophotometer.

10.) Fill another conical with DI water and auto-zero the spectro at 460 nm:

-Turn on machine.

-Select 'Spectra Manager' -> 'Time Management'

-Place cuvette with water in spectro.

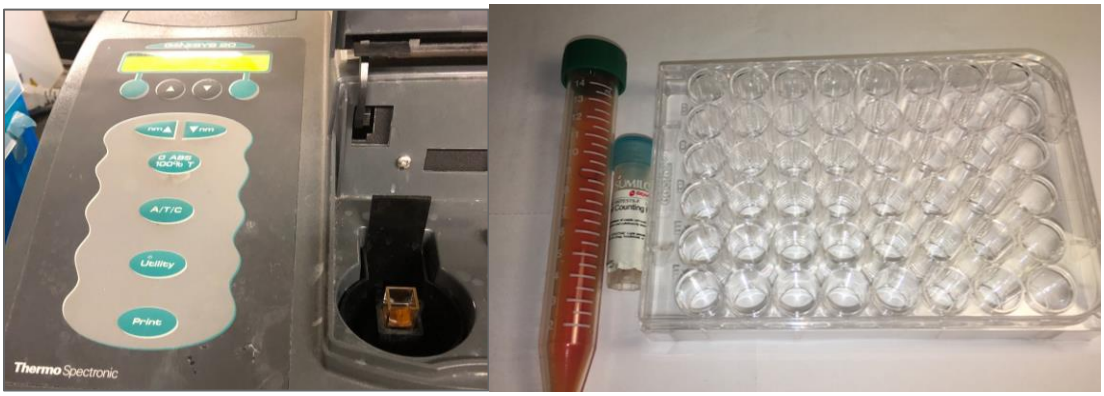
-Select 'Parameters' -> 'Auto-zero', and enter 460 nm for wavelength.

11.) Empty conical into a clean and dry cuvette being sure not to introduce any bubbles. (fig.1)

12.) Take a ten second measurement of the sample and record average absorbance.

13.) Repeat steps 11-12 for each sample.

Figure 1: Spectrophotometer, well plate, CCK product, and conical with sample used for NIH testing.



<u>NIH-3T3 Test Results</u>

Results from the first NIH run were not used. The cells proliferated so much that each well exceeded the recommended working range for the assay, which is 5000-50000 cells per well. For the next run, the cells were plated only two days before testing. Example results from the program as well as absorbance values from the test can be seen below. Results from this test were as expected: absorbance of media with CCK assay increases linearly with cell quantity.

Figure 2: NIH-3T3 assay results. Both screenshots show results for highest 100000 cells/well. True average absorbance can be seen at the top left of the screenshot in red text.

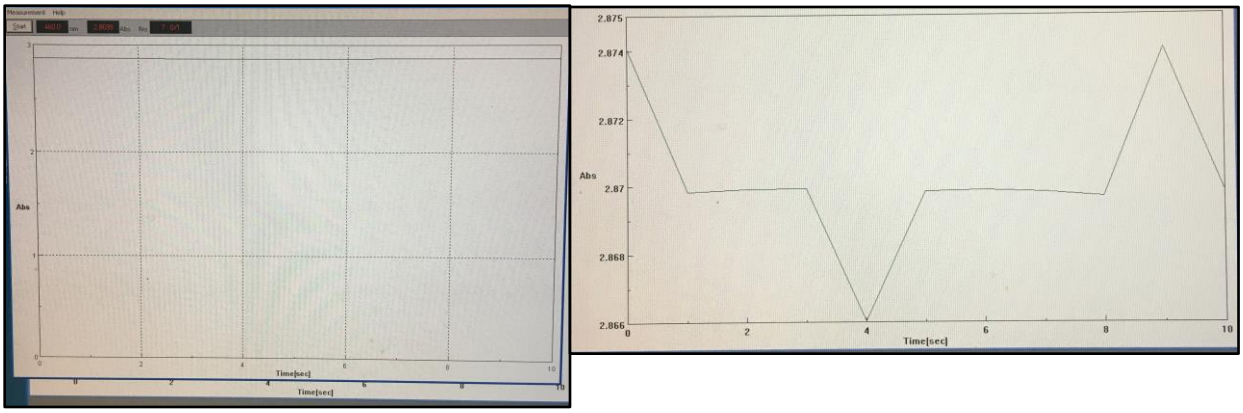
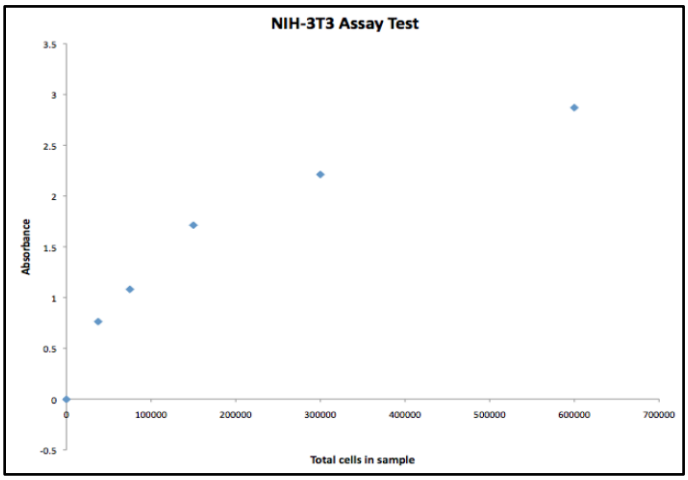


Table 8: All NIH test results.

Cells/well	Total cells in 3.0 mL sample	Absorbance
Control (CCK in DI water)	0	-0.003
6250	37500	0.763
12500	75000	1.081
25000	150000	1.713
50000	300000	2.212
100000	600000	2.870



Significance: PASS, CCK-8 cellular assay proved to be accurate in determining cellular proliferation based on absorbance values at 460 nm.

Scaffold Testing

Equipment: our device, PLGA scaffolds, 48 well plate, bone cells, nutrient media, CCK-8 assay, micropipette, gloves, 15 mL conicals, DI water
Location and time: 192-328 (plating and incubation) and 33-394 (absorbance measurement) on Week 9.
Procedure:
1.) Obtain all equipment and sterilize if necessary.
2.) Seed cells onto scaffolds according to the following:
-Five scaffolds will be used for each of our five test groups, totaling 25 scaffolds.
-There are four prong designs and one control group with no prongs, totaling five groups.
-Scaffolds must be placed in the wells on the plate that correspond to a specific prong design on our device.
-Each scaffold will have 25000 cells.
3.) Once plated, pipette 0.60 mL nutrient media into each well with scaffolds.
4.) Cover well plate with our device and place in incubator at 37°C, 5% CO_2 for 28 hours.
5.) Remove plate from incubator, remove lid, and add 30 microliter of CCK-8 cellular assay to each well with scaffolds and media.
6.) Place well plate in incubator for 3.5 hours. Remove well plate.
7.) For wells of the same prong design, pool each of the five wells into a 15 mL conical using a pipette. You should now have five 15 mL conicals each with 3.0 mL of sample.
8.) Cover the conicals with tinfoil and take them to the spectrophotometer.
9.) Fill another conical with DI water and auto-zero the spectro at 460 nm:
-Turn on machine.
-Select ‘Spectra Manager’ -> ‘Time Management’
-Place cuvette with water in spectro.
-Select ‘Parameters’ -> ‘Auto-zero’, and enter 460 nm for wavelength.
10.) Empty one conical into a clean and dry cuvette being sure not to introduce any bubbles.
11.) Take a ten second measurement of the sample and record average absorbance.
12.) Repeat steps 10-11 for each sample.

Scaffold Test Results

Table 9: All scaffold test results, with our device.

Design	Number of Small Prongs	Base diameter (mm)	Prong diameter (mm)	Absorbance
1	3	5	1.5	2.870
2	3	5	2	2.334
3	5	7	1.5	2.413
4	6	7	2	2.164
5 (Control)	-	-	-	1.533

Significance: PASS, our device was effective in promoting cellular proliferation as opposed to a normal well plate lid.

Autoclave Testing

The autoclave testing was performed to ensure the device was sterilizable and reusable per the customer specifications matrix. The autoclave testing was performed in 192-328 and Cardinal's Lab. The equipment required was an autoclave, autoclave bag, indicator tape and calipers. Safety training was needed for access to 192-328. There was no training needed for use of the autoclave in Cardinal's Lab, since Cardinal's lab assistants ran the autoclave for us. The protocol performed for the autoclave testing is outlined below.

1. Measure the distance between prongs for each of the 48 well inserts. Use calipers to measure the distance between the outside surfaces of two prongs. For the three pronged prong type, three measurements for prong distance should be taken. The measurement technique is shown in (A). For the five pronged prong type, two measurements should be taken for the prong distance(B) and two measurements should be taken for the six pronged prong type(C).
2. Measure the diameter of the prong base for each well insert of the device.
3. Measure the width, length and thickness of the top of the device
4. The device was then placed in a bag and sealed. Indicator tape was placed on the top of the bag(C). The autoclave was set to run at a temperature of 210 degrees celsius for 6 minutes.
5. After autoclaving, the indicator strip color was examined to determine if an acceptable temperature for sterilization had been reached(D).
6. The same measurement taken in steps 1-3 were then performed again.
7. The measurements were then uploaded into minitab.
8. A Turkey Comparison Test with a 95% confidence interval was performed to compare the prong distance, prong base and top(width,length and thickness) measurements before and after autoclaving.

9. The measurements from autoclave testing are shown in Appendix H. The statistical analysis is also shown below in Figures 2-4. There were no significant differences found for distance between prongs, prong base diameter and top dimensions before and after autoclaving for all of the four prong types.

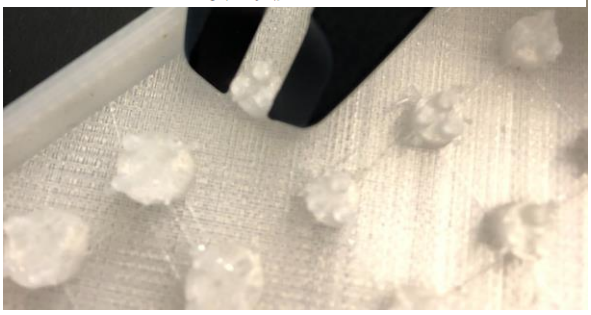
Comparisons for C2

Tukey Pairwise Comparisons: Subscripts

Grouping Information Using the Tukey Method and 95% Confidence

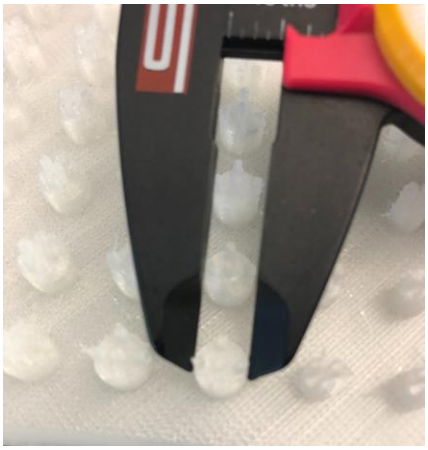
Subscripts	N	Mean	Grouping
5 Prong 1.5mm Diameter After	24	6.84167	A
5 Prong 1.5mm Diameter Before	24	6.80833	A

Means that do not share a letter are significantly different.

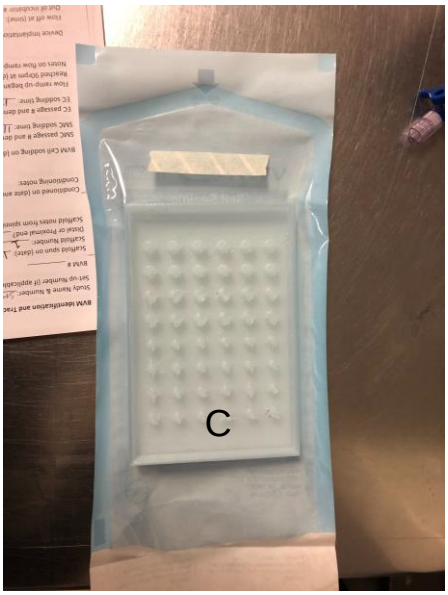


A

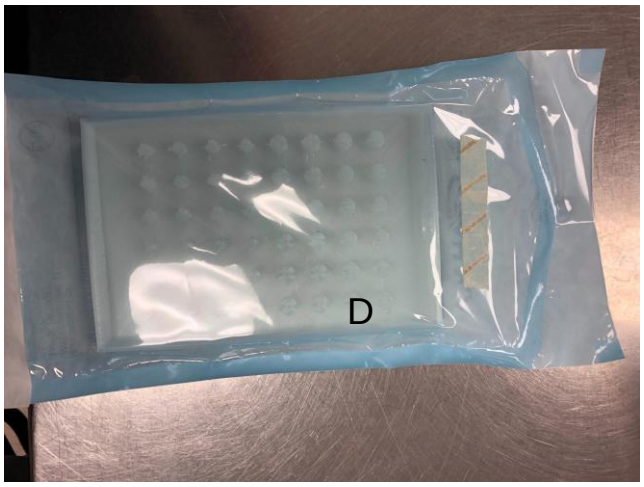
Figure 3: Autoclave testing measurement processes. (C) & (D) show the sterilization bag used.



B



A

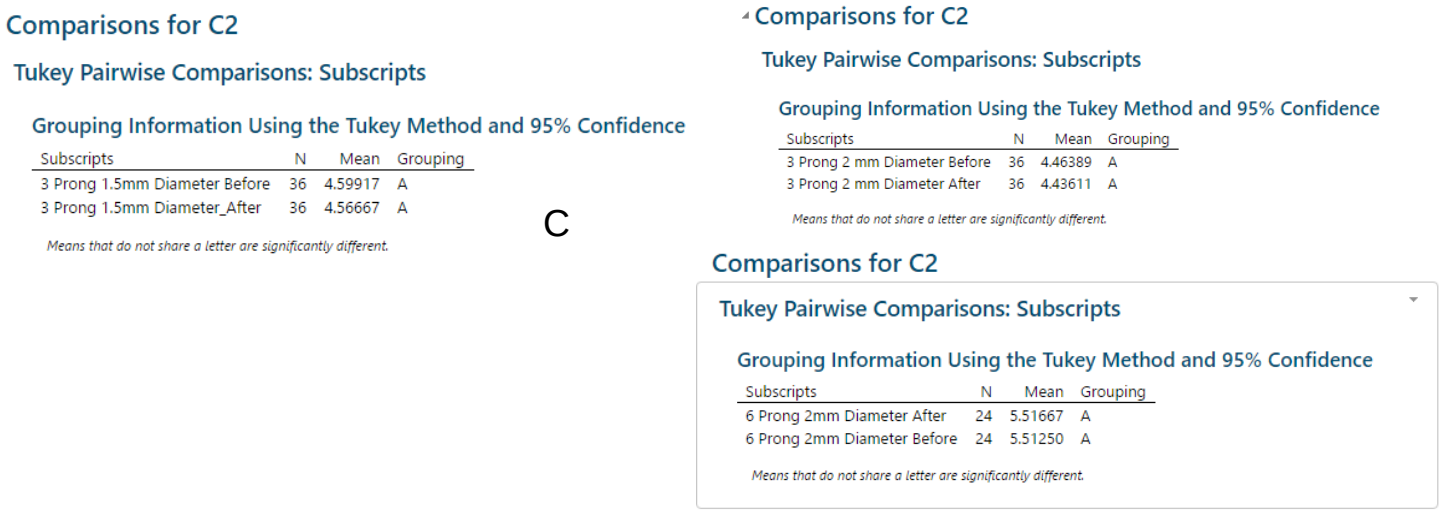


B

C

D

Figure 4: Anova Tukey Comparison testing with a 95% confidence interval for the distance



between prongs for each prong type.(A) Statistical analysis for the three prong 1.5 mm diameter prong type.(B) Statistical Analysis for the three prong 2mm diameter prong type.(C) Statistical Analysis for the five prong 1.5 mm diameter prong type.(D) Statistical Analysis for the six prong 2mm diameter prong type.

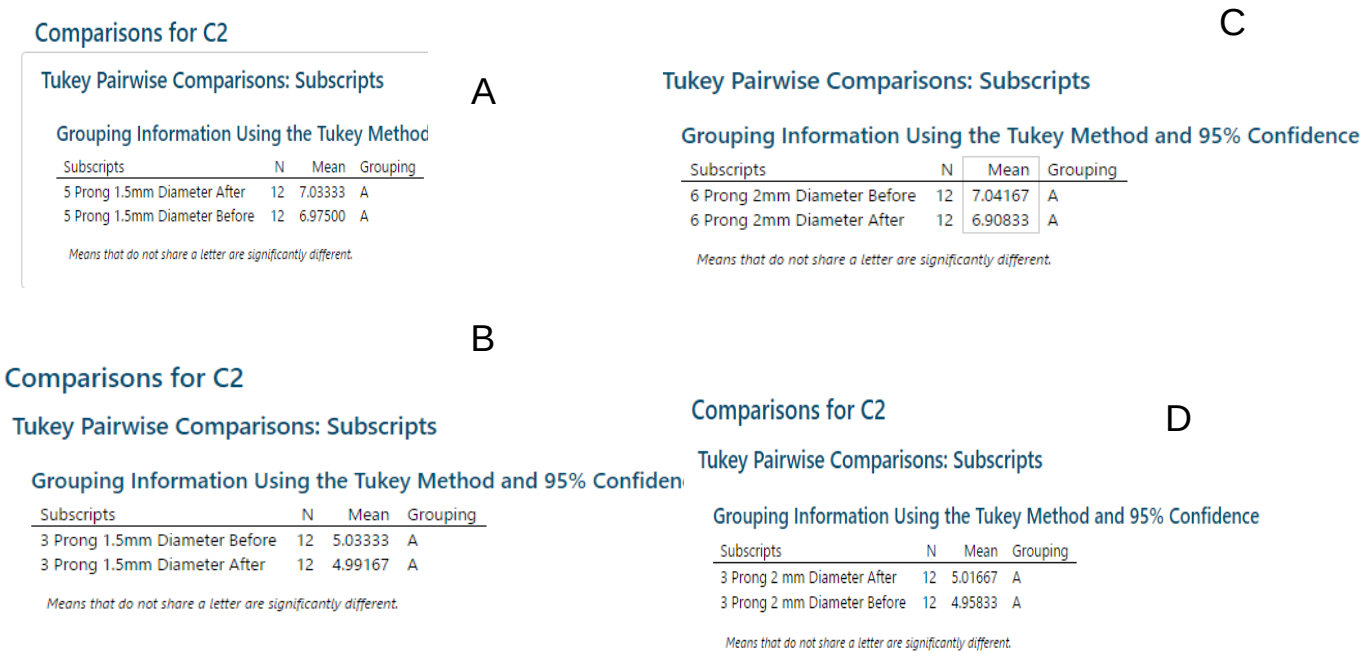


Figure 5: Anova Tukey Comparison testing with a 95% confidence interval for the diameter of the prong base for each prong type.(A) Statistical analysis for the three prong 1.5 mm diameter prong type.(B) Statistical Analysis for the three prong 2mm diameter prong type.(C) Statistical

Analysis for the five prong 1.5 mm diameter prong type.(D) Statistical Analysis for the six prong 2mm diameter prong type.

Tukey Pairwise Comparisons: Subscripts

Grouping Information Using the Tukey Method and 95% Confidence

Subscripts	N	Mean	Grouping
After	3	71.4500	A
Before	3	71.3133	A

Means that do not share a letter are significantly different.

*Figure 6:*Anova Tukey Comparison testing with a 95% confidence interval for the top dimension of the device(width, length and thickness).

Significance: PASS, autoclave sterilization at 230°C for 5 minutes did not significantly warp or alter the dimensions of our device.

Incubation Testing

Incubation testing will be completed to ensure the dimensions of the device do not significantly change during incubation. Testing for incubation testing is performed in 192-328. The equipment needed is an incubator, well media, well plate and calipers. Safety training was need for access to 192-328. The protocol for the incubation testing is outlined below.

1.) Measure the distance between each prong for each of the 48 well inserts. Calipers are used to measure the distance between the outside surfaces of two prongs. For the three pronged prong type, three measurements for prong distance should be taken. The measurement technique is shown in Figure 2. For the five pronged prong type, two measurements should be taken for the prong distance(B) and two measurements should taken for the six pronged prong type(C).

2.) Measure the diameter of the prong base for each well insert of the device.

3.) Fill wells with cell media.

4.) Place device on top of well plate.

5.) Incubate for 72 hours at 37°C, 5% CO_2.

6.) Perform steps 1-3 again.

7.) Perform a Tukey Comparison Test with a 95% confidence interval to compare the prong distance, prong base and top(width,length and thickness) measurements before and after autoclaving.

The measurements from incubation testing are shown in Appendix I. The statistical analysis is also shown below in Figures 7 and 8. There were no significant differences found for distance between prongs, prong base diameter and top dimensions before and after autoclaving for all of the four prong types.

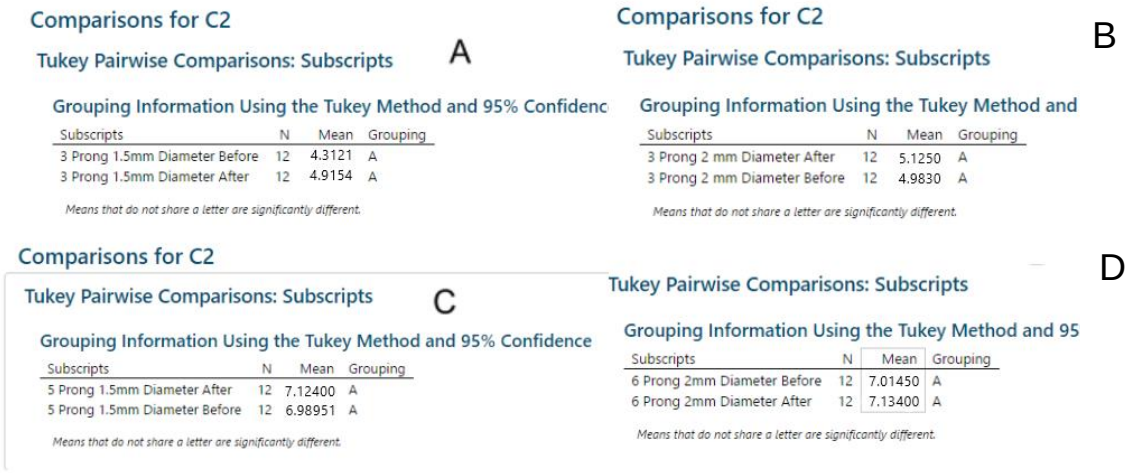


Figure 7: Anova Tukey Comparison testing with a 95% confidence interval for the distance between prongs for each prong type.(A) Statistical analysis for the three prong 1.5 mm diameter prong type.(B) Statistical Analysis for the three prong 2mm diameter prong type.(C) Statistical Analysis for the five prong 1.5 mm diameter prong type.(D) Statistical Analysis for the six prong 2mm diameter prong type.

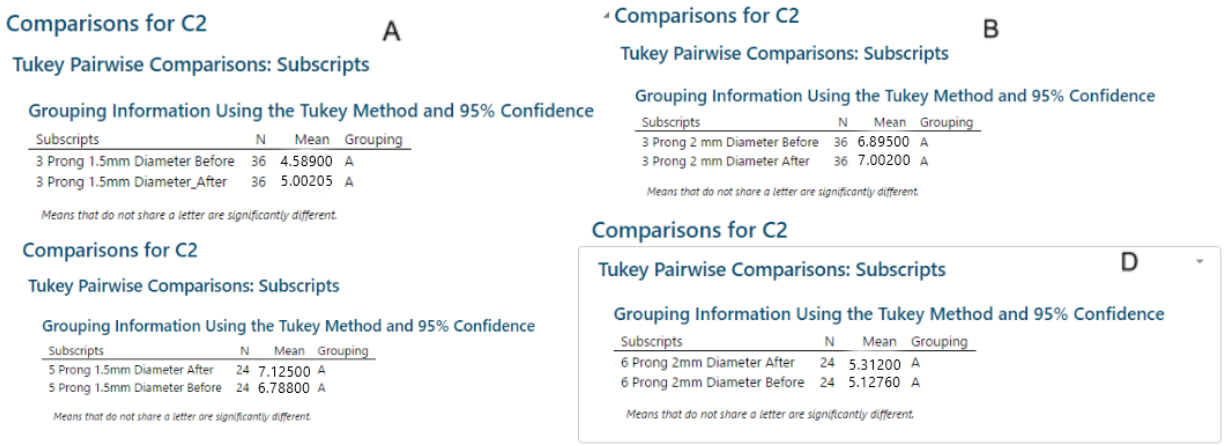


Figure 8: Anova Tukey Comparison testing with a 95% confidence interval for the diameter of the prong base for each prong type.(A) Statistical analysis for the three prong 1.5 mm diameter prong type.(B) Statistical Analysis for the three prong 2mm diameter prong type.(C) Statistical Analysis for the five prong 1.5 mm diameter prong type.(D) Statistical Analysis for the six prong 2mm diameter prong type.

Significance: PASS, incubation at 37°C, 5% CO_2 for 72 hours did not significantly warp or alter the dimensions of our device.

8.0 Conclusions and Recommendations

8.1 Recommendations

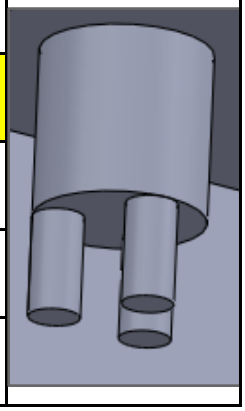
Through trial and error we developed some recommendations for use. First, after printing there will be thin material strings going from prong to prong. These are leftover from printing and may interrupt the fit, since they are not part of the design. These imperfections and other sharp edges melt away during the autoclave process. We recommend our device be sterilized in the autoclave at 210C for at least 6 minutes. We sterilized our device at 260C as well and did not observe differences in dimensions. Once the print is done, we recommend it be left on the print bed for at least ten minutes to allow for complete cooling. When placing on and removing the device from the well plate, each side must be raised simultaneously or the top surface can crack.

8.2 Conclusions

After testing, Nylon 6 proved to be a sterilizable material. Almost all warping comes from the printing process rather than the sterilization process. Slight warping may occur up to five minutes after the print. Warping our our device did not alter the dimensions or affect the fit on the well plate. We autoclaved our device three times and did not observe any dimension variation. Our tip design also proved effective. As long as the minimum prong diameter was 1.5 mm, no deformation in prongs was observed during sterilization or incubation. All prongs on our

device generated more cell growth versus the control, with prong design #1 being the clear 'winner'. Our final device will be reprinted with uniform prongs of the following dimensions:

Table 10: DOE Results to Determine Best Prong Choice

Prong Design	Number of Small Prongs	Base diameter (mm)	Prong diameter (mm)	
1	3	5	1.5	
2	3	5	2	
3	5	7	1.5	
4	6	7	2	

9.0 Acknowledgments

We would like to especially thank the following individuals for their contributions to our project:

- Dr. Christopher Heylman for sponsorship, professional guidance and feedback
- Dr. Michael Whitt for professional guidance and feedback
- Theo Anastos for tissue culture testing
- Conor Hadigan for autoclave sterilization assistance
- Dr. Michael Black for lab access
- Dr. Kirsten Cardinal for lab access
- QL+ Lab officer, Craig Icban, for manufacturing assistance

10.0 Appendices

10.1 Appendix A: References

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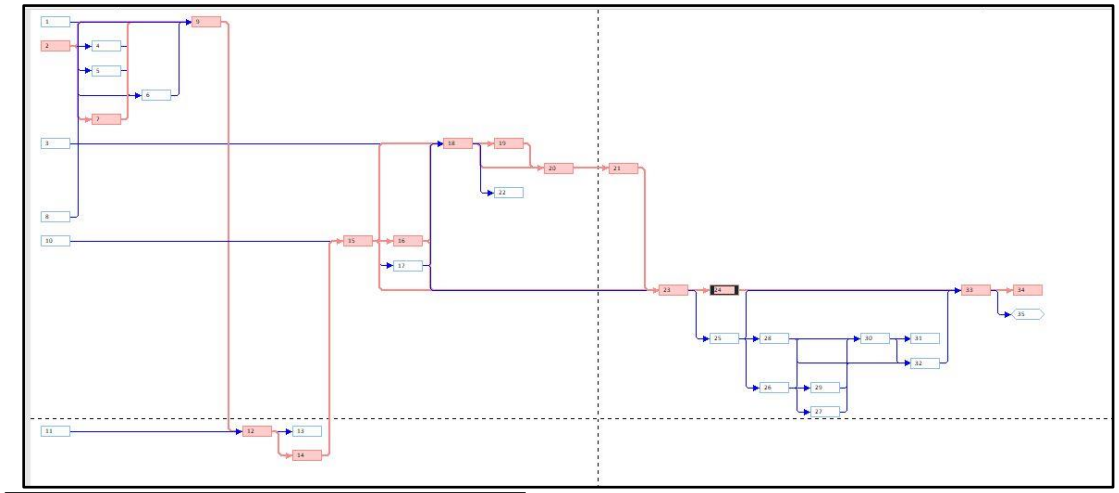
10.2 Appendix B: Project Plan (PERT Chart)

Figure 9: PERT chart and legend from Microsoft Project.



	Task Mode	Task Name	Duration	Start	Finish	Predecessors
1		IFU Statement	3 days	Mon 10/1/18	Wed 10/3/18	
2		Product Specification Matrix	3 days	Mon 10/1/18	Wed 10/3/18	
3		Yellow Tag Cert	23 days	Mon 10/1/18	Wed 10/31/1	
4		Budget Determination	3 days	Thu 10/4/18	Mon 10/8/18	2
5		Hazard and Risk Assesment	3 days	Thu 10/4/18	Mon 10/8/18	2
6		FMEA	2 days	Tue 10/9/18	Wed 10/10/1	2,5
7		DOE	8 days	Thu 10/4/18	Mon 10/15/1	2
8		Conjoint Analysis	3 days	Mon 10/1/18	Wed 10/3/18	
9		Project Planning Presentation	3 days	Tue 10/16/18	Thu 10/18/18	2,7,6,5,4,8,1
10		Intellectual Property	1 day	Mon 10/1/18	Mon 10/1/18	
11		House of Quality	1 day	Mon 10/1/18	Mon 10/1/18	
12		Pugh Chart	2 days	Fri 10/19/18	Mon 10/22/1	9,11
13		Stutus Update Memo 1	4 days	Tue 10/23/18	Fri 10/26/18	12
14		Concept Development	9 days	Tue 10/23/18	Fri 11/2/18	12
15		Concept Review Presentation	3 days	Mon 11/5/18	Wed 11/7/18	10,14
16		Material Selection	11 days	Thu 11/8/18	Thu 11/22/18	15
17		Manufcaturing Plan	9 days	Thu 11/8/18	Tue 11/20/18	15
18		Midpoint Project Report	3 days	Fri 11/23/18	Tue 11/27/18	15,16,17,3
19		Status Update Memo 2	2 days	Wed 11/28/18	Thu 11/29/18	15,18
20		Design Freeze Presentation	6 days	Fri 11/30/18	Fri 12/7/18	18,19
21		Preliminary Prototype Presentation	5 days	Mon 12/10/18	Fri 12/14/18	20
22		First Draft of Final Report	64 days	Wed 11/28/18	Mon 2/25/19	18
23		Final Test Manufacturing Plan Presentation	3 days	Mon 12/17/18	Wed 12/19/18	17,15,16,21
24		Preliminary Functional Prototype Presentation	65 days	Thu 12/20/18	Wed 3/20/19	23
25		Order Material	1 day	Mon 1/14/19	Mon 1/14/19	23
26		Spectrophotometer Calibration	1 day	Thu 1/24/19	Thu 1/24/19	25
27		Order Assay Kits	1 day	Fri 1/25/19	Fri 1/25/19	26
28		3D Print	3 days	Fri 1/25/19	Tue 1/29/19	25
29		Final Test and Manufacturing Plans Presentation	1 day	Fri 1/25/19	Fri 1/25/19	26
30		First Run of Proliferation Tests	7 days	Wed 1/30/19	Thu 2/7/19	26,28,27
31		Autoclave and Incubation Testing	4 days	Fri 2/8/19	Wed 2/13/19	30
32		Status Update Memo IV	5 days	Fri 2/8/19	Thu 2/14/19	28,29,30
33		Final Design and Prototype Presentation	5 days	Thu 3/21/19	Wed 3/27/19	24,25
34		Final Poster Presentation	3 days	Thu 3/28/19	Mon 4/1/19	33
35		Final Report	0 days	Mon 3/18/19	Mon 3/18/19	22,26

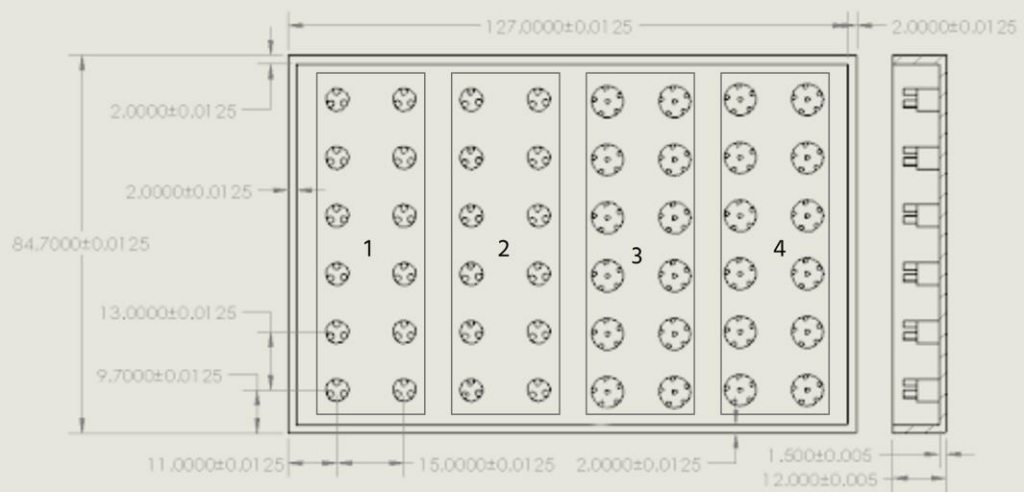
10.3 Appendix C: CAD Drawings

All dimensions in millimeters. The differences between types 1 & 2 and between 3 & 4 are base prong and small prong lengths, diameters are the same.

Table 11: Specific prong type designs.

Prong Design	Number of Small Prongs	Base diameter (mm)	Prong diameter (mm)
1	3	5	1.5
2	3	5	2
3	5	7	1.5
4	6	7	2

Figure 6: SolidWorks drawing of device, all dim's in mm.



10.4 Appendix D: FMEA, Hazard & Risk Assessment

Our testing provided some valuable insight on mitigating risks while manufacturing our device. Though we only performed autoclave testing at the required temperature, we did sterilize our device at various temperatures. Significant deformation was seen when sterilizing at 260°C for 15 minutes. Sterilization should be performed at either 210°C for 15 minutes or at 230°C for five minutes only. 1.5 mm diameter proved to be the best small prong design. This design is able to be efficiently manufactured, but the prongs can be broken off fairly easily by human hands. For this reason, the small prongs on the underside of the device should never be touched, unless absolutely necessary. All sharp or potentially dangerous edges on our device are nicely smoothed out during sterilization. The autoclave eliminates any left over, free hanging strands of material as well.

Table 12: Risks and Hazards Assessment table.

Hazard	Planned Corrective Action
Small and sharp edges on device	Fillet design edges, machine down to smooth
Small prongs can break off	Enlarge prong diameter
Material toxicity during incubation	Change material
Extreme incubation environment	Change material or treat material surface prior to use
Prongs fail to submerge scaffolds	Change prong length

Table 13: FMEA Assessment.

Component Name	Possible Failure Mode	Type	Cause of Failure	OCC	DET	SEV	RPN	Effect of Failure on System	Failure Improvement Alternative Actions (actions to fix the problem…)	Comments
Base	Fracture	C,M	Degradation of mechanical properties due to incubation and continuous use, parts not properly or fully joined	1	3	3	9	Depending on level of breakage the scaffolds could be moved and cell proliferation could decrease causing the scaffolds to be	Determine a lifespan for the product,ensure the material chosen has desirable mechanical properties for the lab environment.	

								useless.		
Prongs	Bending	M	Unable to bear load of device	1	2	6	12	Whole device could shift and spill the cell medium.The scaffolds could also be damaged.	Choose a size,shape and material that will support the load of the device.	
Prong	Degradation,Fracture	M	Incubation could cause degradation of the mechanical properties material and potentially a fracture.	3	4	6	72	Failure of the prong could result in perforation of the scaffold and for the scaffold to not be submerged in the cell medium.	Choose a material with desirable mechanical properties for the lab environment.	
Material of Body and Prongs	Toxicity, Expansion	M	Incubation could lead to toxic particles from the material interaction with the cell medium or expansion of the parts.	4	1	9	32	Toxicity could affect the growth of the cells. Expansion of the components could lead to movement of the device or fracture.	Choose a material with a low coefficient of thermal expansion. Choose a material that is biocompatible.	
Material of Tip	Mechanical stress on cells	M	Damage the scaffolds, negatively affect cell growth	3	1	9	18	Reacting with the scaffolds could lead to changes in cell growth	Choose an inert material and create a testing protocol to ensure it doesn't negatively affect cell growth.	

10.5 Appendix E: Pugh Chart

Concept 1: Well plate lid with base and prongs projecting into wells
Concept 2: Semipermeable membrane/cage housing scaffolds
Concept 3: Bioreactor completely separate from well plate

Table 14: All pugh charts. Concept ideas seen above.

Selection Criteria	**CellCrown 24 NX Well Inserts**	**Concept 1**	**Concept 2**	**Concept 3**
Lifetime	Datum	+	+	+
Stability		S	-	S
Contact Area		+	+	+
Biocompatibility		S	S	S
Price per Unit		+	S	-
Cell Growth and Proliferation		+	S	+
Manufacture Difficulty		+	+	-
# Pluses	n/a	5	3	3
# Minuses	n/a	0	1	2

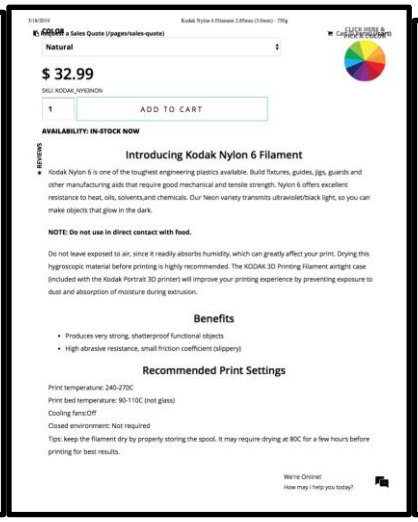
Selection Criteria	**Concept 1**	**Concept 2**	**Concept 3**
Lifetime	Datum	S	+
Stability		-	-
Contact Area		-	+
Biocompatibility		S	S
Price per Unit		+	-
Cell Growth and Proliferation		S	+
Manufacture Difficulty		S	-
# Pluses	n/a	1	3
# Minuses	n/a	2	3

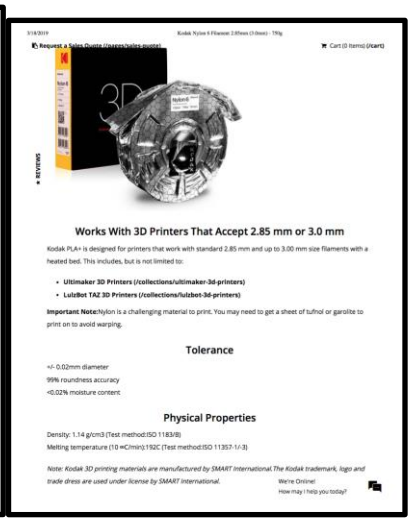
Selection Criteria	Concept 2	Concept 1	Concept 3
Lifetime	Datum	S	+
Stability		+	+
Contact Area		+	+
Biocompatibility		S	S
Price per Unit		S	-
Cell Growth and Proliferation		S	+
Manufacture Difficulty		S	-
# Pluses	n/a	2	4
# Minuses	n/a	0	2

Selection Criteria	Concept 3	Concept 1	Concept 2
Lifetime	Datum	-	-
Stability		+	S
Contact Area		-	-
Biocompatibility		S	S
Price per Unit		+	+
Cell Growth and Proliferation		-	-
Manufacture Difficulty		+	+
# Pluses	n/a	3	2
# Minuses	n/a	3	3

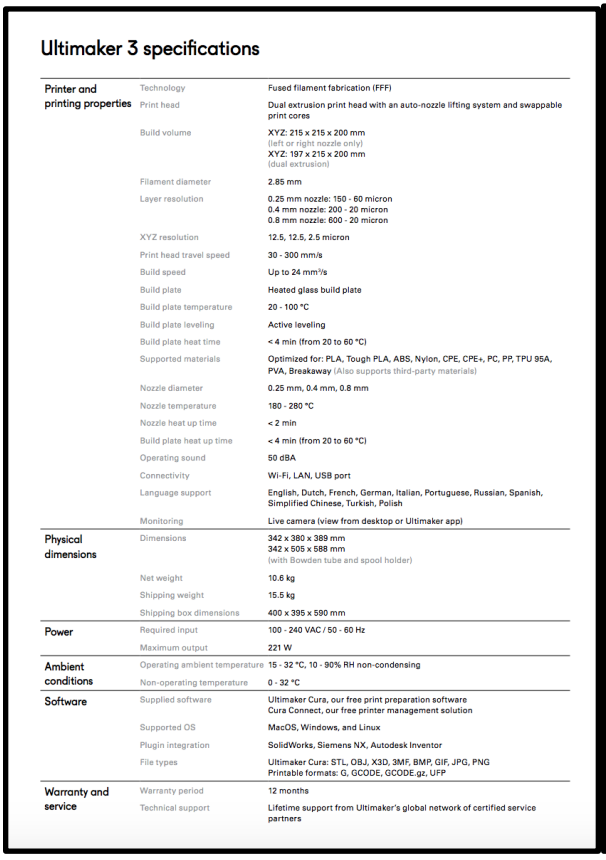
10.6 Appendix F: Vendor Information, Specifications, and Data Sheets

Nylon 6 material:



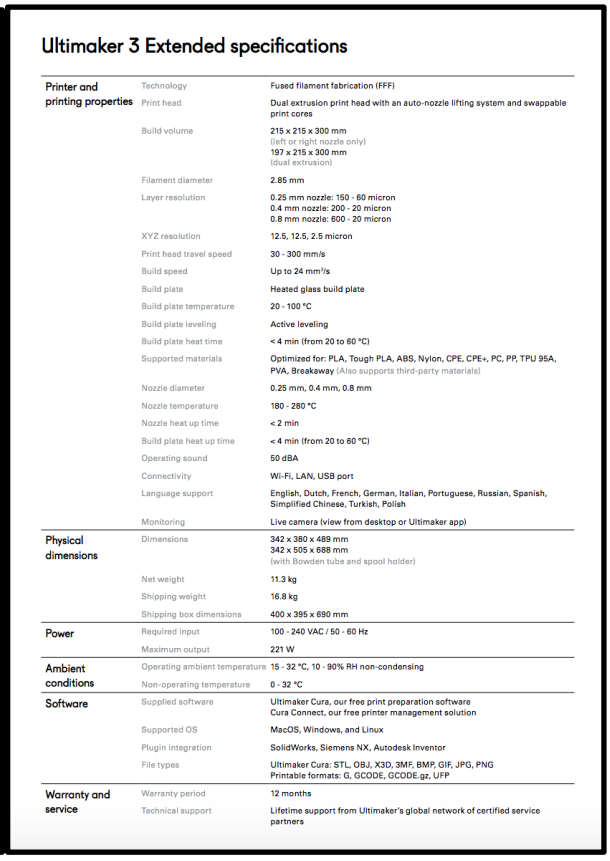


Ultimaker 3D Printer:



Ultimaker 3 specifications

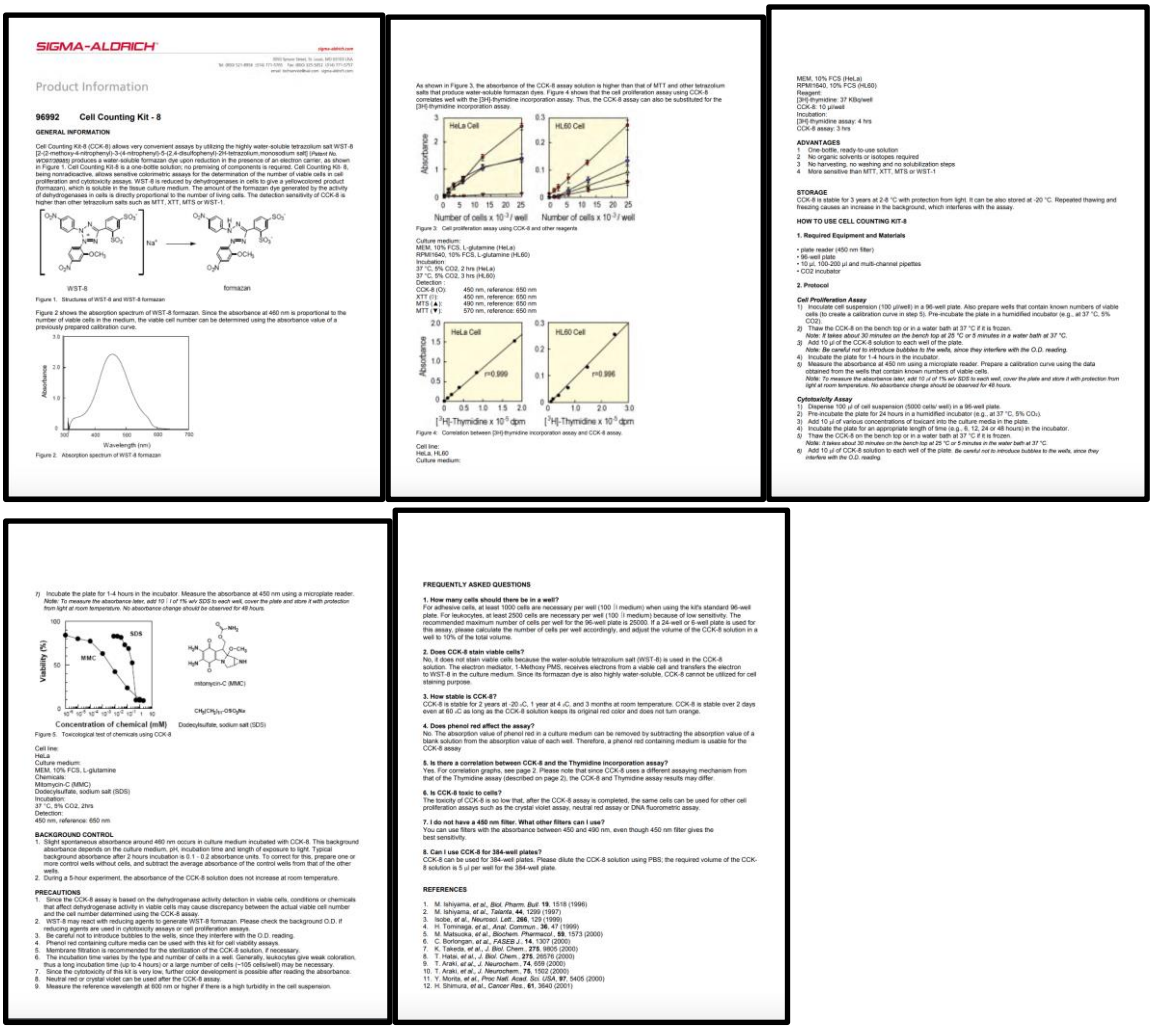
Printer and printing properties	Technology	Fused filament fabrication (FFF)
	Print head	Dual extrusion print head with an auto-nozzle lifting system and swappable print cores
	Build volume	XYZ: 215 x 215 x 200 mm (left or right nozzle only) XYZ: 197 x 215 x 200 mm (dual extrusion)
	Filament diameter	2.85 mm
	Layer resolution	0.25 mm nozzle: 150 - 60 micron 0.4 mm nozzle: 200 - 20 micron 0.8 mm nozzle: 600 - 20 micron
	XYZ resolution	12.5, 12.5, 2.5 micron
	Print head travel speed	30 - 300 mm/s
	Build speed	Up to 24 mm³/s
	Build plate	Heated glass build plate
	Build plate temperature	20 - 100 °C
	Build plate leveling	Active leveling
	Build plate heat time	< 4 min (from 20 to 60 °C)
	Supported materials	Optimized for: PLA, Tough PLA, ABS, Nylon, CPE, CPE+, PC, PP, TPU 95A, PVA, Breakaway (Also supports third-party materials)
	Nozzle diameter	0.25 mm, 0.4 mm, 0.8 mm
	Nozzle temperature	180 - 280 °C
	Nozzle heat up time	< 2 min
	Build plate heat up time	< 4 min (from 20 to 60 °C)
	Operating sound	50 dBA
	Connectivity	Wi-Fi, LAN, USB port
	Language support	English, Dutch, French, German, Italian, Portuguese, Russian, Spanish, Simplified Chinese, Turkish, Polish
	Monitoring	Live camera (view from desktop or Ultimaker app)
Physical dimensions	Dimensions	342 x 380 x 389 mm 342 x 505 x 588 mm (with Bowden tube and spool holder)
	Net weight	10.6 kg
	Shipping weight	15.5 kg
	Shipping box dimensions	400 x 395 x 590 mm
Power	Required input	100 - 240 VAC / 50 - 60 Hz
	Maximum output	221 W
Ambient conditions	Operating ambient temperature	15 - 32 °C, 10 - 90% RH non-condensing
	Non-operating temperature	0 - 32 °C
Software	Supplied software	Ultimaker Cura, our free print preparation software Cura Connect, our free printer management solution
	Supported OS	MacOS, Windows, and Linux
	Plugin integration	SolidWorks, Siemens NX, Autodesk Inventor
	File types	Ultimaker Cura: STL, OBJ, X3D, 3MF, BMP, GIF, JPG, PNG Printable formats: G, GCODE, GCODE.gz, UFP
Warranty and service	Warranty period	12 months
	Technical support	Lifetime support from Ultimaker's global network of certified service partners



Ultimaker 3 Extended specifications

Printer and printing properties	Technology	Fused filament fabrication (FFF)
	Print head	Dual extrusion print head with an auto-nozzle lifting system and swappable print cores
	Build volume	215 x 215 x 300 mm (left or right nozzle only) 197 x 215 x 300 mm (dual extrusion)
	Filament diameter	2.85 mm
	Layer resolution	0.25 mm nozzle: 150 - 60 micron 0.4 mm nozzle: 200 - 20 micron 0.8 mm nozzle: 600 - 20 micron
	XYZ resolution	12.5, 12.5, 2.5 micron
	Print head travel speed	30 - 300 mm/s
	Build speed	Up to 24 mm³/s
	Build plate	Heated glass build plate
	Build plate temperature	20 - 100 °C
	Build plate leveling	Active leveling
	Build plate heat time	< 4 min (from 20 to 60 °C)
	Supported materials	Optimized for: PLA, Tough PLA, ABS, Nylon, CPE, CPE+, PC, PP, TPU 95A, PVA, Breakaway (Also supports third-party materials)
	Nozzle diameter	0.25 mm, 0.4 mm, 0.8 mm
	Nozzle temperature	180 - 280 °C
	Nozzle heat up time	< 2 min
	Build plate heat up time	< 4 min (from 20 to 60 °C)
	Operating sound	50 dBA
	Connectivity	Wi-Fi, LAN, USB port
	Language support	English, Dutch, French, German, Italian, Portuguese, Russian, Spanish, Simplified Chinese, Turkish, Polish
	Monitoring	Live camera (view from desktop or Ultimaker app)
Physical dimensions	Dimensions	342 x 380 x 489 mm 342 x 505 x 688 mm (with Bowden tube and spool holder)
	Net weight	11.3 kg
	Shipping weight	16.8 kg
	Shipping box dimensions	400 x 395 x 690 mm
Power	Required input	100 - 240 VAC / 50 - 60 Hz
	Maximum output	221 W
Ambient conditions	Operating ambient temperature	15 - 32 °C, 10 - 90% RH non-condensing
	Non-operating temperature	0 - 32 °C
Software	Supplied software	Ultimaker Cura, our free print preparation software Cura Connect, our free printer management solution
	Supported OS	MacOS, Windows, and Linux
	Plugin integration	SolidWorks, Siemens NX, Autodesk Inventor
	File types	Ultimaker Cura: STL, OBJ, X3D, 3MF, BMP, GIF, JPG, PNG Printable formats: G, GCODE, GCODE.gz, UFP
Warranty and service	Warranty period	12 months
	Technical support	Lifetime support from Ultimaker's global network of certified service partners

CCk-8 assay:



10.7 Appendix G: Budget

Table 15: Budget Outline for Project

Product	**Distributor**	**Cost**	**Unit**	**Amount**	**Details**	**Product Number**	**For**
Nylon 6	3D Universe	40.49	each	1	750 g spool of 2.85 mm filament	KODAK_NY63NON	Manufacturing
Garolite sheet	McMaster Carr	10.68	each	1	6" x 6" 1/32" thick	9910T59	Manufacturing

CCK-8	Sigma	64	each	3	100 test pack	96992	Proliferation testing
Food coloring	Target	3.95	each	1	dropper bottle red dye	-	Proliferation testing
		247.12					

*Garolite sheet was not used. The slightly different Garolite sheet listed in 5.4 is the proper one necessary for future manufacturing.

10.8 Appendix H: Autoclave Raw Data

Table 14: Distance Between Prongs Before Autoclave

Distance Between Prongs Before Autoclave(mm)			
3 Prong 1.5mm Diameter	3 Prong 2 mm Diameter	5 Prong 1.5mm Diameter	6 Prong 2mm Diameter
4.57	4.3	6.5	5.5
4.5	4.5	6.7	5.7
4.5	4.5	6.5	5.5
4.4	4.4	6.7	5.6
4.7	4.5	7	5.5
4.5	4.5	6.2	5.7
4.8	4.4	8	5.6
6.5	4.5	7	5.5
6	4.5	7.1	5.6
4.5	4.3	6.8	5.5
4.4	4.4	6.7	5.2
4.5	4.5	6.5	5.5
4.6	4.6	6.7	5.2
4.4	4.3	7.1	5.4
4.8	4.5	6.9	5.3
4.4	4.3	6.7	5.4
4.5	4.5	7	5.4
4.3	4.5	6.5	5.5
4.3	5	7	5.8
4.5	5	6.1	5.5
4.4	4.5	7	5.3
4.5	4.4	7.1	5.6
4.5	4.4	6.7	6
4.6	4.5	6.9	5.5
4.7	4.3		
4.4	4.4		
4.6	4.5		
4.5	4.1		
4.4	4.2		
4.5	4.3		
4.5	4.5		
4.6	4.3		
4.4	4.6		
4.5	4.7		
4.4	4.5		
4.4	4.5		

Table 15: Base Diameter of each Prong Before Autoclave

Base Diameter of Each Prong Before Autoclave(mm)			
3 Prong 1.5mm Diameter	3 Prong 2 mm Diameter	5 Prong 1.5mm Diameter	6 Prong 2mm Diameter
5	4.9	6.8	7
5	4.9	6.9	7
5.1	5	6.8	7.1
5	5	6.9	6.8
5	5	7.2	6.8
5	4.9	6.9	7
5.2	5.2	6.9	6.9
5	4.9	6.9	7
5	5	6.8	7
5.2	4.8	6.7	7
4.9	4.9	6.9	6.9
5	5	6.9	8

Table 16: Measurements of the Top of the Device Before Autoclave

Measurements of Top Of Device Before Autoclave(mm)	
Width	85.1
Thickness	1.4
Length	127.44

Table 17: Distance Between Prongs After Autoclave

Distance Between Prongs After Autoclave(mm)			
3 Prong 1.5mm Diameter	3 Prong 2 mm Diameter	5 Prong 1.5mm Diameter	6 Prong 2mm Diameter
4.5	4.3	6.8	5.8
4.5	4.3	6.7	5.1
4.7	4.6	6.8	5.3
5	4.3	7.4	5.5
4.4	4.7	6.5	5.7
4.5	4.6	6.5	5.9
4.5	4	6.9	5.3
4.4	4.3	6.8	5.7
4.4	4.7	6.9	5.7
5.3	4.6	6.8	5.5
5.2	4.7	6.7	5.7
4.5	4.5	6.7	5.6
4.5	4.5	6.7	5.1
4.5	4	6.5	5.5
4.7	4.2	6.7	5.1
4.3	4.3	6.9	5.4
4.4	4.3	6.7	6
4.4	4.5	6.7	5
4.5	4.2	7.3	5.4
4.3	4.1	6.9	5.5
4.4	4.7	8	5.5
4.1	4.2	6.5	5.8
4.5	4.4	7.2	5.7
4.7	4.6	6.6	5.6
4.3	4.9		
4.3	4.4		
4.7	4.4		
5	4.3		
4.3	4.1		
4.5	4.4		
4.9	4.4		
4.9	4.4		
5	4.3		
4.4	5.7		
4.4	4.3		
4.5	4.5		

Table 18: Base Diameter of Each Prong After Autoclave

Base Diameter of Each Prong After Autoclave(mm)			
3 Prong 1.5mm Diameter	3 Prong 2 mm Diameter	5 Prong 1.5mm Diameter	6 Prong 2mm Diameter
4.9	4.9	7	6.8
4.9	5	7	6.8
4.9	5	7	6.8
5	5.1	7.1	6.8
5.1	5.1	7.1	7.1
4.9	5,1	7.1	6.9
5	5	7	6.6
5	4.9	6.9	6.9
5	4.9	7.2	7.1
5	5	7.1	6.9
5.1	5.1	7.1	6.9
5.1	5.1	7	7.3

Table 19: Measurements of the Top of the Device After Autoclave

Measurements of Top Of Device After Autoclave(mm)	
Width	85.84
Thickness	1.41
Length	127.1

10.9 Appendix I:Incubation Raw Test Data

Table 20: Distance Between Prongs Before Incubation

Distance Between Prongs Before Incubation(mm)			
3 Prong 1.5mm Diameter	3 Prong 2 mm Diameter	5 Prong 1.5mm Diameter	6 Prong 2mm Diameter
4.5	4.4	6.5	5.4
4.5	4.6	6.7	5.4
4.6	4.4	7.1	5.5
4.7	4.5	6.9	5.8
4.4	4.1	6.7	5.5
4.6	4.2	7	5.7
4.5	4.3	8	5.6
4.4	4.5	7	5.5
4.5	4.5	7.1	5.6
4.5	4.3	6.8	5.5
4.4	4.4	6.7	5.2
4.5	4.5	6.5	5.5
4.6	4.6	6.7	5.2
4.4	4.3	7.1	5.4
4.8	4.5	6.9	5.3
4.4	4.3	6.7	5.4
4.5	4.5	7	5.4
4.3	4.5	6.5	5.5
4.3	5	7	5.8
4.5	5	6.1	5.5
4.4	4.5	7	5.6
4.5	4.4	7.1	5.5
4.5	4.4	6.7	5.2
4.6	4.5	6.9	5.5
4.7	4.3		
4.4	4.4		

Table 21: Base Diameter of Each Prong Before Incubation

Base Diameter of Each Prong Before Incubation(mm)			
3 Prong 1.5mm Diameter	3 Prong 2 mm Diameter	5 Prong 1.5mm Diameter	6 Prong 2mm Diameter
5	5	6.7	7
5	4.8	6.9	6.9
5.2	4.9	6.9	7
5	5	6.9	6.8
5	5.1	7.2	6.8
5	4.9	6.8	7
5.2	5.2	6.9	6.9
5	4.9	7.2	7
5	5	6.8	7
5.2	4.7	6.7	7
4.9	4.9	6.9	6.9
5	5	6.9	7.5

Table 22: Measurements of the Top of the Device Before Incubation

Measurements of Top Of Device Before Incubation(mm)	
Width	85.2
Thickness	1.3
Length	127.44

Table 23: Distance Between Prongs After Incubation

Distance Between Prongs After Incubation(mm)			
3 Prong 1.5mm Diameter	3 Prong 2 mm Diameter	5 Prong 1.5mm Diameter	6 Prong 2mm Diameter
4.5	4	6.7	5.7
4.7	4.3	6.5	5.7
4.3	4.7	6.7	5.5
4.3	4.7	6.9	5.7
4.4	4.7	6.5	5.7
4.5	4.6	6.5	5.9
4.5	4	6.9	5.3
4.4	4.3	6.8	5.7
4.4	4.7	6.9	5.7
5.3	4.6	6.7	5.5
5.2	4.7	6.9	5.7
4.5	4.5	6.7	5.1
4.5	4.6	6.7	5.4
4.5	4	7.3	6
5.3	4.3	6.9	5
5.2	4.7	8	5.4
4.5	4.6	6.5	6
4.4	4	6.7	5
4.5	4.3	7.3	5.4
4.3	4.7	6.9	5.5
4.4	4.7	8	5.1
4.1	4.2	6.5	5.4
4.5	4.4	7.2	6
4.7	4.6	6.6	5
4.3	4.9		
4.3	4.4		

Table 24: Base Diameter of Each Prong After Incubation

Base Diameter of Each Prong After Incubation(mm)			
3 Prong 1.5mm Diameter	3 Prong 2 mm Diameter	5 Prong 1.5mm Diameter	6 Prong 2mm Diameter
5	5.1	7.1	6.8
5	5	7.1	6.8
5	4.9	7.1	7.1
5	5.1	7	6.9
5.1	5	7.1	7.1
4.9	4.9	7.1	6.9
5	4.9	7.1	6.9
4.9	5	6.9	6.6
5	4.9	7.2	6.9
5	5	7.1	6.9
5.1	5.1	7.1	6.9
5.1	5.1	7	7.3

Table 25: Measurements of the Top of the Device After Incubation

Measurements of Top Of Device After Autoclave(mm)	
Width	85.75
Thickness	1.44
Length	127.5