

SUMMER RESEARCH

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Project Objectives

- Design of high-throughput TRACER platform that compatible with the 96 well-plate formats (with design and construction)
- Characterization of various parts of the device (custom well plate, scaffolds, syringe holder, seeding device/pipeline)
- Expansion design on smaller (384 well plate) design for other studies (and find possible uses of the design)
- Stacking 6 layers of scaffold which have regions blocked off PMMA

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Component/progress

A: 3D printing of the 96-well PMMA disk

Demonstrating Proof of Concept(G-CODE)

Trial#	Description	Imaging
1		
2	4mm diameter 4 layers 0.5mm spacing	
3	3000 speed	

*Note: The PMMA used is the old one Jose made. The PMMA concentration is 17.5% weight/volume

Treatment #	radius(mm)	# of Layers	Layer Spacing(mm)	Speed(mm/s)
1	3.25	2	0.25	3000
2	3.0			
3	3.25		0.5	
4	3.0			
5	3.25	3	0.25	1000
6	3.0			
7	3.25		0.5	
8	3.0			
9	3.25	4	0.25	3000
10	3.0			
11	3.25		0.5	
12	3.0			
13	3.25	2	0.25	3000
14	3.0			
15	3.25		0.5	
16	3.0			
17	3.25	3	0.25	1000
18	3.0			
19	3.25		0.5	
20	3.0			
21	3.25	4	0.25	3000
22	3.0			
23	3.25		0.5	
24	3.0			

Table A2. Various parameter testing

PRINT PATTERN CHARACTERIZATION/RESULT

PMMA Formula B

- 1.75mg of PMMA
- 1.0mg of methyl Blue
- 7.84mL of 100% Acetone
- Instructions for production:
- Mass and combine all components, shake overnight
- Store in fridge for remainder of usage

Experiments

First Round Trial

Speed	D=6.5mm d=0.25mm 2LAY	D=6.0mm d=0.25mm 2LAY	D=6.5mm d=0.5mm 2LAY	D=6.0mm d=0.5mm 2LAY	D=6.5mm d=0.25mm 3LAY	D=6.0mm d=0.25mm 3LAY
3000						
1000						
Speed	D=6.5mm d=0.5mm 3LAY	D=6.0mm d=0.5mm 3LAY	D=6.5mm d=0.25mm 3LAY	D=6.0mm d=0.25mm 3LAY	D=6.5mm d=0.5mm 4LAY	D=6.0mm d=0.5mm 4LAY
3000						
1000						

Figure A2 – Prints for tA1-14 with PMMA formula B

Final Parameters (denoted as tB1):

3.00mm inner radius
0.5mm ring spacing
4 rings per well
3000mm/ms printing speed

Second Round Trial

Figure A3. tA10 and tA12 5-8 layers prints with PMMA formula B
(File GCODE_6mm_5-8LAY.gcode ; GCODE_6mm_6LAY.mlx ; GCODE_6mm_8LAY.mlx)

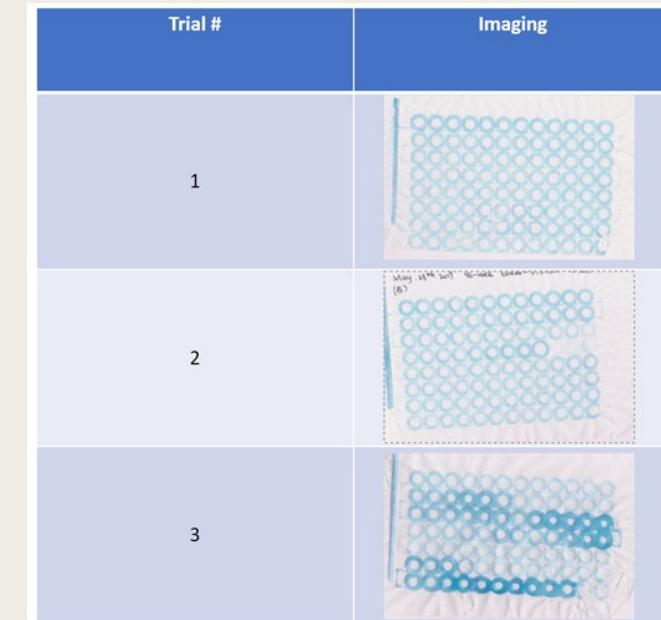
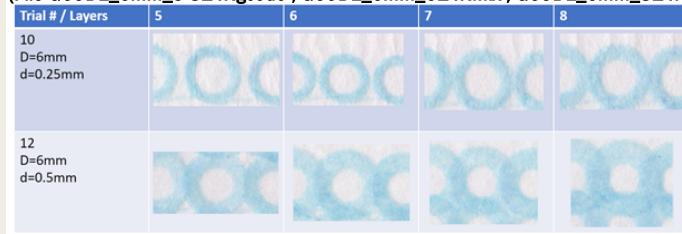


Figure A4. Final Print tB1 with PMMA formula B (GCODE_6mm_4LAY.mlx ; GCODE_6mm_4LAY.gcode)

Image Analysis

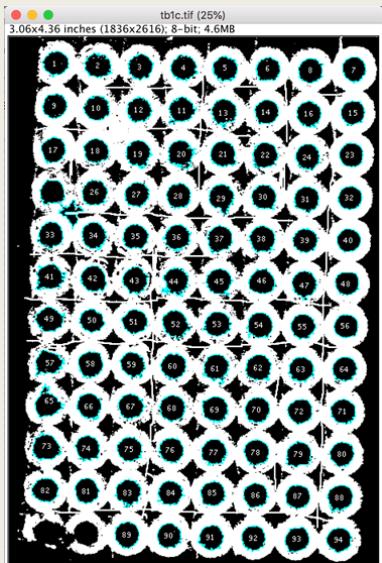
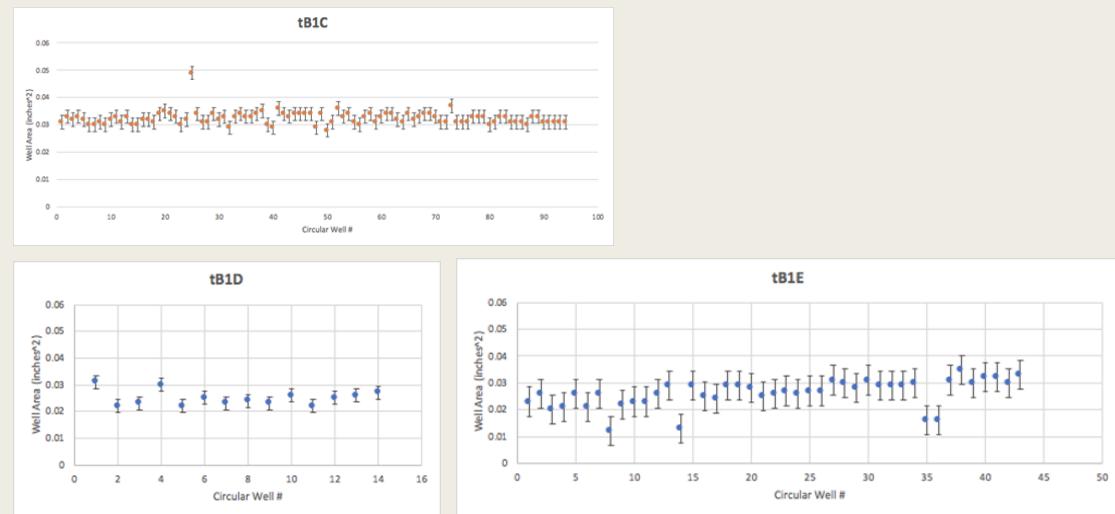


Figure A5. tb1 imaged print

Table A3: Average well areas for 96-well PMMA-printed circles computed in inches².

Print	# of wells taken into account	Average well area (inches ²)
tB1c	94	0.03241489
tB1d	14	0.02492857
tB1e	43	0.02616279

Figure A6. Well Area Distribution for tB1C, tB1D, tB1E



Discussion

- ***Leakage of PMMA Formula A***
- ***Presence of air bubbles in syringe tips***
- ***Damage of printing paper from adhesive tape***
- ***Lowered Platform***

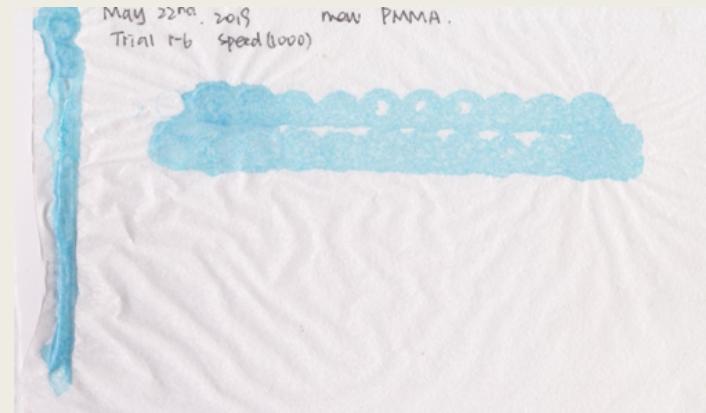


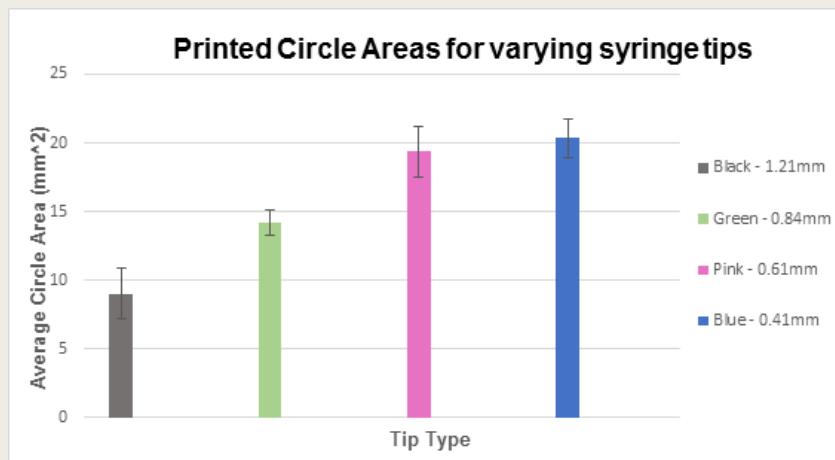
Figure A7 – tA1-6 print with Formula A (low viscosity), May22

Tip selection/Characterization

Figure A8: Cropped images of prints for varying tip sizes. (File tips_testing.gcode)



Figure A9: Data summarizing printed circle areas for varying tip sizes. Standard deviation calculated from replicates. [1]



Discussion

■ *Lowered Platform*

- Used the screws and springs beneath the platform to adjust the platform height
- Scrapped out some excess PMMA from the syringe holder

■ *Presence of air bubbles in syringe tips*

- Reduction in air bubble issues after switching out the bottom portion of the syringe for a new piece

■ *Leakage of PMMA Formula A (volume/volume)*

- Alleviated by switching to *Formula B (weight/volume)*

Dyes selection for visualizing PMMA

- We selected four types of dyes for experiment: methyl blue. Food dye, basic nail polish, no dye.

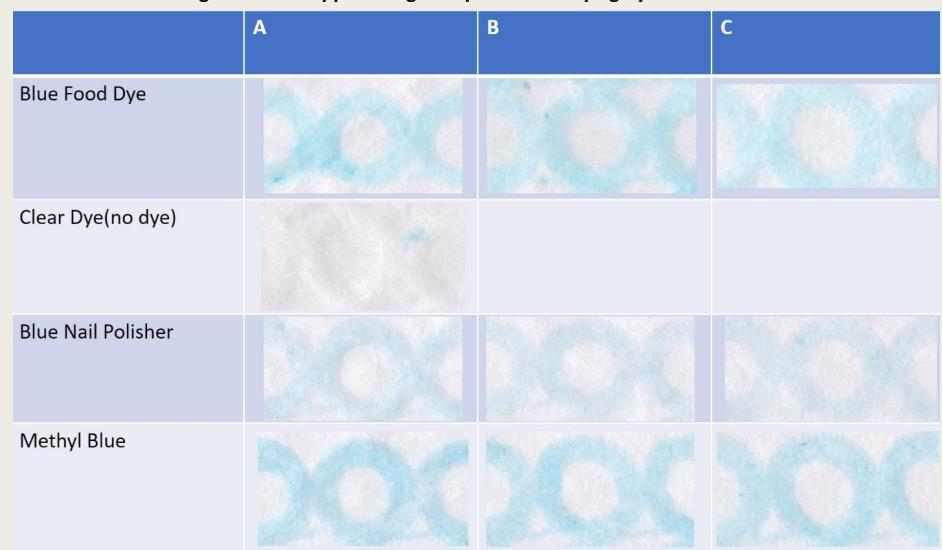
Table A4. Dyes selection and testing protocol

Dye Type	Amount	Print Pattern	Date Printed
Methyl Blue	0.001g	3mm radius, 4 revolutions, 0.5mm	2019-06-25
Food Dye	1 drop	revlotion spacing,	2019-06-25
Basic Nail Polish	1 drop	3000mm/ms	2019-06-25
Clear Coat	1 drop		

Table A5 Image processing result of different dyes

Type of Dye	Trial #	Average Area	Circularity	
Blue Food Dye	A	0.027167	6	0.876
	B	0.025194	31	0.88
	C	0.026914	35	0.979
Clear Dye(no Dye)	A	N/A (can't recognize)	0	N/A
Blue Nail Polisher	A	0.027143	21	0.86
	B	0.028706	17	0.862
	C	N/A (can't recognize)	0	N/A
Mythl Blue	A	0.025618	34	0.878
	B	0.027486	35	0.889
	C	0.026611	36	0.887

Figure A10: Cropped images of prints for varying dyes



Drained with PBS

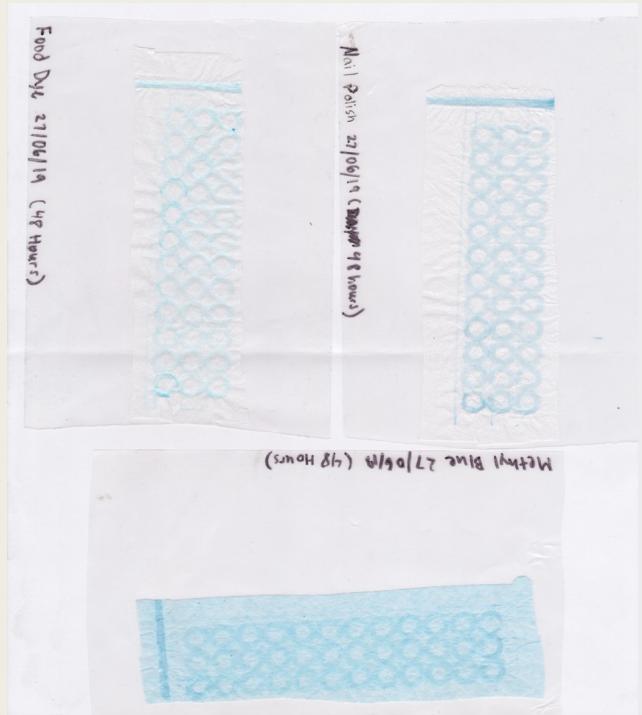


Figure A11: Scanned images of 36 96-well circles (printed in final parameters in Formula) dyed with Methyl Blue, Food dye, and blue Nail Polish after 48hrs in hot water bath with PBS.

Pigment Selection

Written Name	CI Number	CAS Number	McMaster-Carr	VWR	Smallest Mass Available	Price	Form	Cytotoxic?
ALUMINUM LAKE	(CI 19140)	12225-21-7	None	https://ca.vwr.com/store/product/en/9564564/food-yellow-no-4-aluminum-lake	25g	\$ 172.64	Powder	Unknown(VWR)
ALUMINUM POWDER	(CI 77000)	7429-90-5	None	https://ca.vwr.com/store/product?ciNum=7429-90-5	25g	\$42.63	Powder	Unknown(VWR)
MANGANESE VIOLET	(CI 77742)	10101-66-3	None	None	-	-	-	-
D&C RED NO. 6 BARIUM LAKE	(CI 15850)	5858-81-1	None	None	-	-	-	-
IRON OXIDES	(CI 77491, CI 77492, CI 77499)	1309-37-1 / 1345-27-3, 51274-00-1, 12227-89-3 / 1317-61-9 / 1345-27-3	51274-00-1: https://www.mcmaster.com/1347n33	1309-37-1: https://ca.vwr.com/store/product/en/9564564/food-yellow-no-4-aluminum-lake	MMC: 1lb ; VWR: 100g	MMC: \$25.00; VWR: \$22.50	MMC: Powder; VWR: Powder	Unknown(MMC & VWR)
D&C RED NO. 7 CALCIUM LAKE	(CI 15850)	5858-81-1	None	1317-61-9: https://www.mcmaster.com/1347n35	1317-61-9: https://ca.vwr.com/store/product/en/948567/iron-ii-iii-oxide-red-metals-basis	MMC: 1lb; VWR: 1kg	MMC: \$25.00; VWR: \$74.17	MMC: Powder; VWR: Powder
FD&C BLUE NO. 1 ALUMINUM LAKE	(CI 42090)	3844-45-9	None	https://ca.vwr.com/store/product/en/9564556/acid-blue-9	25g	\$154.69	Powder	Unknown(VWR)
FERRIC AMMONIUM FERROCYANIDE	(CI 77510)	14038-43-8 / 12240-15-2 / 25869-00-5	None	14038-43-8: https://ca.vwr.com/store/product/en/9883281/ammonium-iron-ii-hexacyanoferrate-ii-hydrate-tech	25g	\$83.44	Unclear	Unknown(VWR)
D&C RED NO. 34 CALCIUM LAKE	(CI 15880)	6417-83-0	None	None	-	-	-	-
ULTRAMARINES	(CI 77007)	12769-96-9 / 1302-83-6 / 57455-37-5	None	12769-96-9: https://ca.vwr.com/store/product/en/9881274/carbon-black-99-9-acetylene-100-compressed	250g	\$71.27	Unclear	Unknown(VWR)
D&C BLACK NO. 2	(CI 77266)	1333-86-4 / 7440-44-0	None	1333-86-4: https://ca.vwr.com/store/product/en/9880098/carbon-black-99-9-acetylene-50-compressed	250g	\$80.03	Unclear	Unknown(VWR)
				7440-44-0: https://ca.vwr.com/store/product/en/8869550/charcoal	50g	\$10.00	Charcoal, Activated Carbon	Unknown(VWR)

- Since the cytotoxic is unknown for each pigment (McMaster-Carr and VWR were unable to provide cytotoxicity information for any of the pigments isolated from the nail polish), the final recommendation is made based on price.
- Final Recommendations: - FD&C BLUE NO. 1 ALUMINUM LAKE (CAS#: 3844-45-9) from VWR; - IRON OXIDES(CAS#: 1317-61-9) from VWR; - D&C Black No.2 (CAS#: 1333-86-4) from VWR

- Live/dead (cytotoxicity)

The resulted images are not clear enough due to the excess volume of gel added in the samples.

B: 384-well characterization

Table B1. Different treatment group for characterization of 384-well

Treatment #	side length(mm)	# of Layers	Layer Spacing(mm)	Speed(m m/s)	Printed On:	# of Trials Printed
1	3	3	0.5	3000	2019-07-22	4
2	3	5	0.3		2019-07-22	4
3	3.3	3	0.4		2019-07-22	4
4	3.3	5	0.24		2019-07-22	4
5	3.7	3	0.27		2019-07-22	4
6	3.7	5	0.16		2019-07-22	4
7	4.5	1	0		2019-07-23	3
8		3	0		2019-07-23	3
9		5	0		2019-07-23	3
10	FULL(5)	3			2019-07-23	3
11	FULL(6)	5			2019-07-23	3

*Note: 6*6 layer (1,3,5) size(3-4.5); 40 prints from 11 treatment groups were printed on July 22nd and 24th, All prints were scanned and image-analyzed through ImageJ (July 24 and 25)

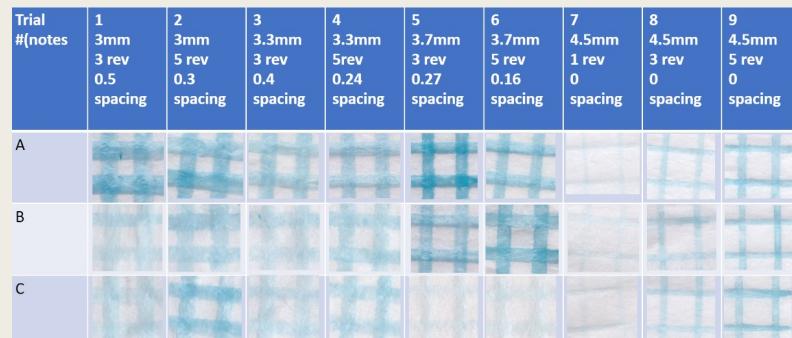
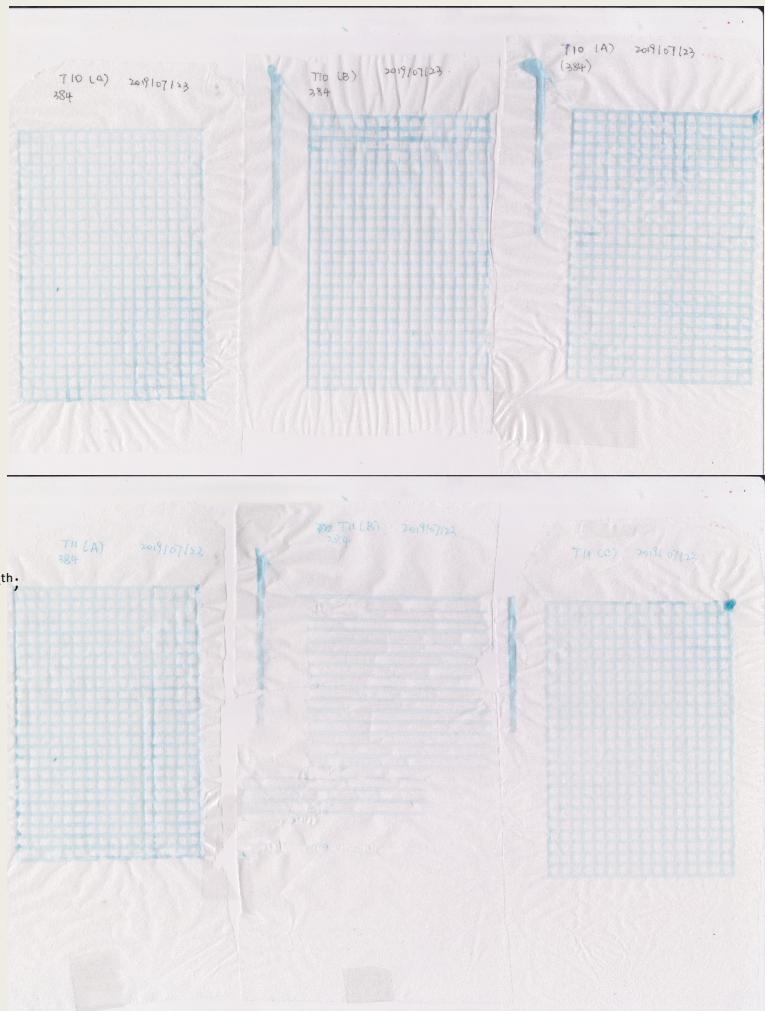


Figure B2. 384 FULL PRINT FOT T5-T6 (File GCODE_LINE_T10 – T11.gcode)



Results

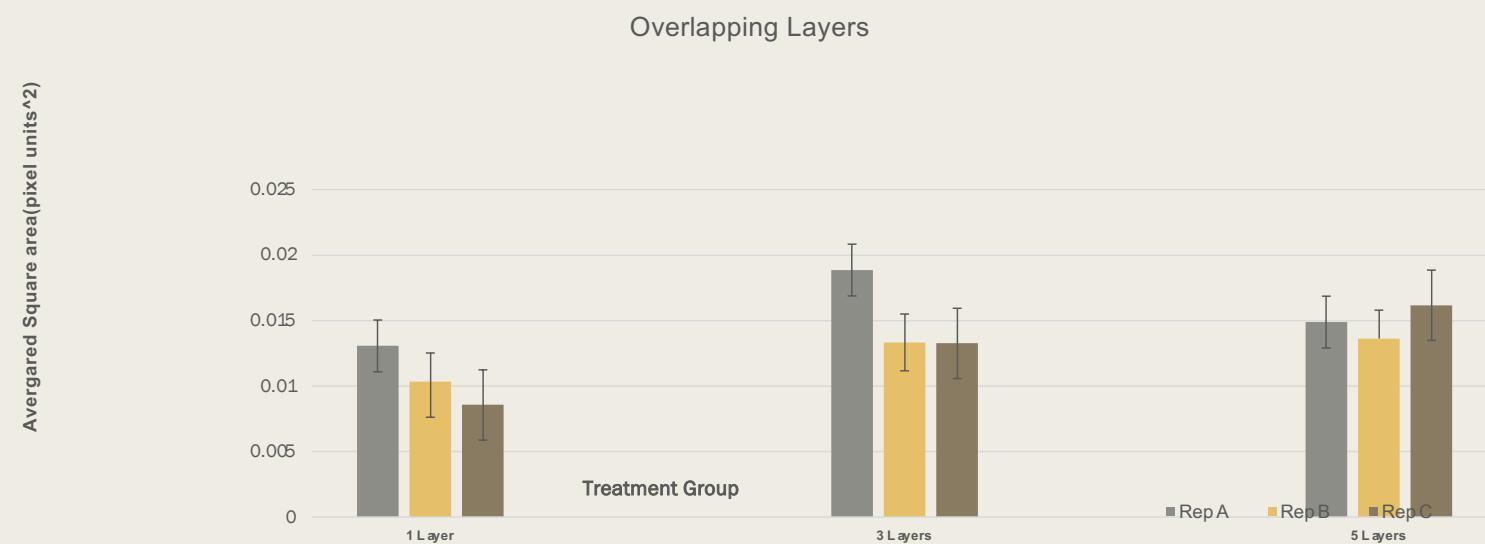


Figure B3: Square area in pixel unit² for 1, 3, and 5-layer overlay of Formula B PMMA pigment on a 6 by 6 grid for a 384 well plate. Each treatment group shows 3 replicates and their standard deviation.

C: Stacked 96-well plate Design Stage

	Dimension(length by width/mm)	Top Height(mm)	Bot Height(mm)	Skirt(mm)	Screw Diameter(mm)	Characteristics
V1	140.71*98.43(inner: 127.65*85.43)	9.5	14.6	8.1		1.9 The top part is covered with a frame and thick bottom
V3	140.71*98.43(inner: 127.71*85.43)	10.6	22	none(2mm embed)		1.9 One edge is cut to indicate direction
V4	123.82*81.48 (inner: 113.95*71.48)	11.1	23.8	none(2mm embed)		1.9 same thickness of actual well plate; two edges are cut; size adjustment in order to use same lid of the 96 well plate
V5	127.76*85.48(with skirt)123.82*81.48; inner:114.6*72.14	11.1(1.58 gasket)	19.3	1.58(2.5mm embed)		1.9 in order to use same lid of the 96 well plate
Final	127.76 * 85.48(inner:115.31*73)	11.1(1.58 gasket)	11.1	2.11(1.58 embed)		2.4 Screw Diameter adjusted to be consistent with the paper; height is shortened for it to observe under microscope

•Comparison

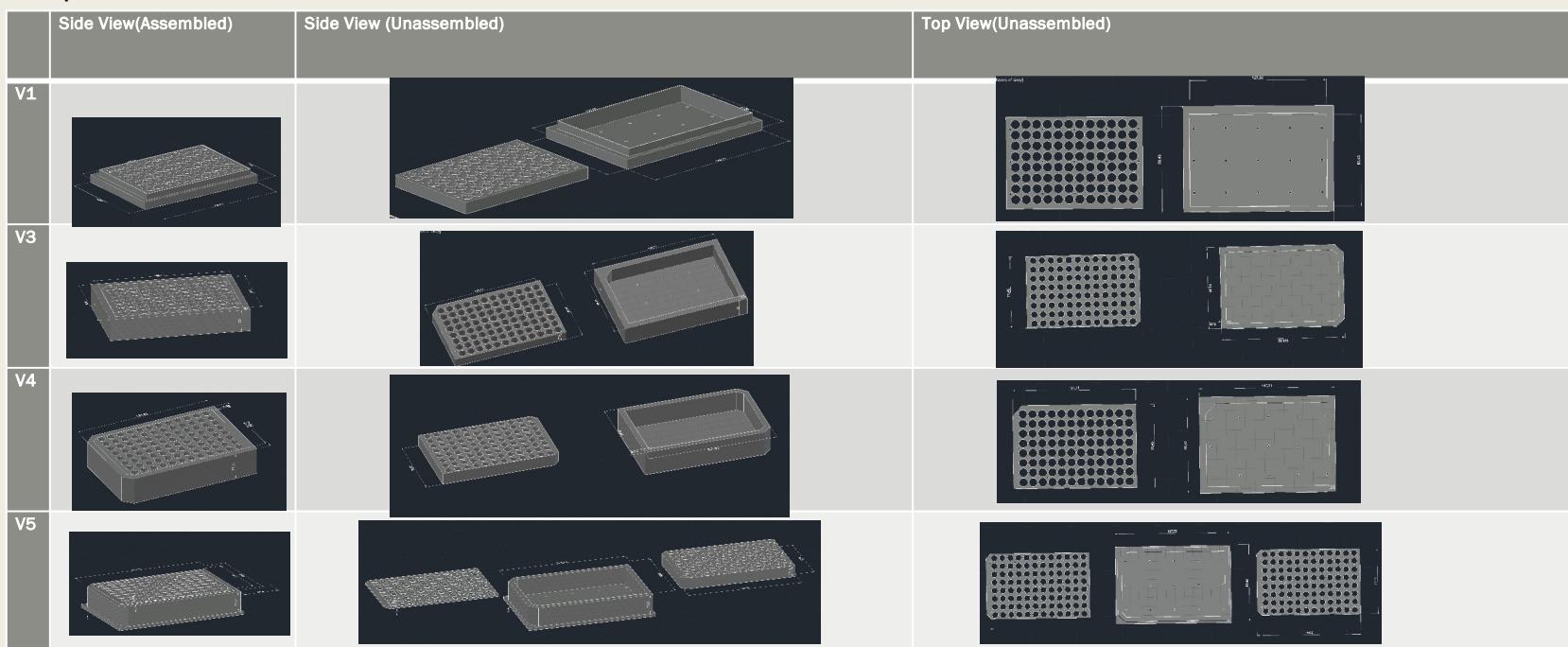
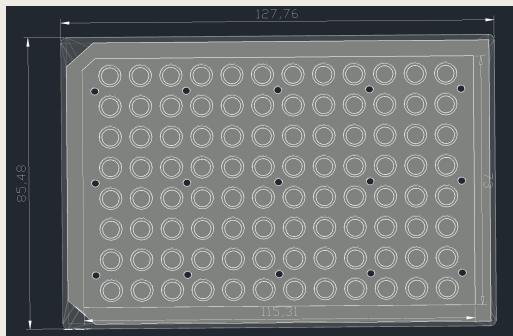


Table C2 comparison between different designs

Final Design

Top view (assembled/unassembled)

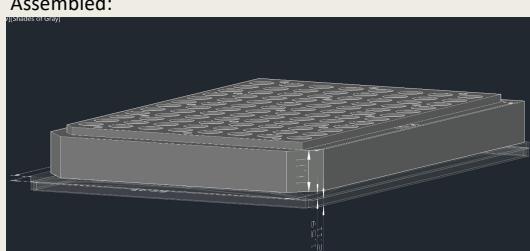


Bot Dimension: 127.76mm * 85.48mm
Top Dimension: 115.31mm*73mm

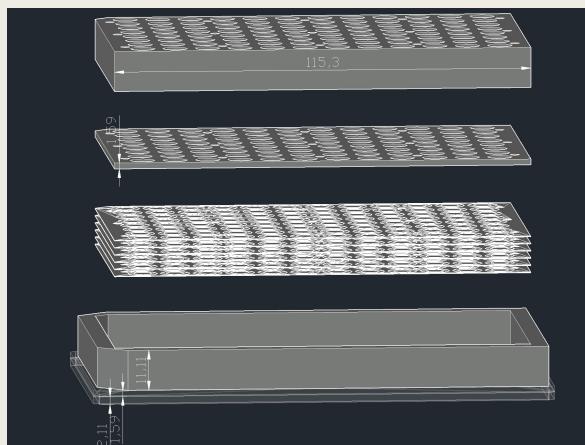
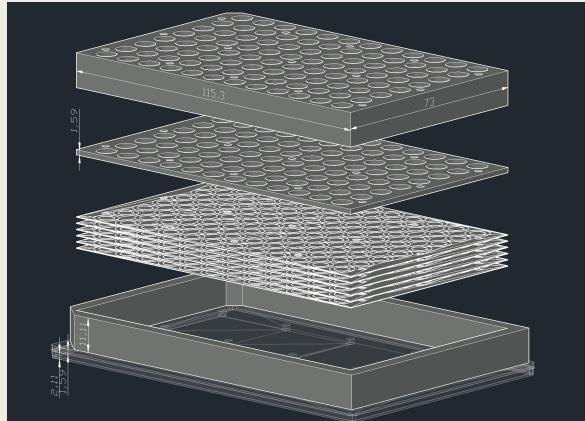
Well Diameter: 6.96mm
Well spacing: 9mm
Screw Diameter:2.4mm

Side view

Assembled:



Bot height: 11.11mm + 1.59mm thickness + 2.11mm skirt
Unassembled:



Gasket:115.3mm*73mm*1.59mm
The designed pieces are being laser cut and assembled with 70% isopropanol

Going Forward:

D: Syringe holder

E: Seeding scaffold pipeline

Gel optimization (2 µL,2.5 µL,3 µL)

Rationale: Current gel volume used in LIVE/DEAD tests (5.0 µL) to check cytotoxicity near interfaces is too large to get a viable image from the microscope

Treatment Groups

Treatment 1:

2.0 µL

Treatment 2:

2.5 µL

Treatment 3:

3.0 µL

Procedure:

- Seed cells with the varying gel concentrations in the PMMA wells

- Allow cells to incubate in wells for 48hrs

- Image PMMA with microscope

Table E1. Seeding Protocol

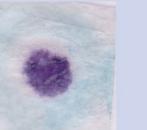
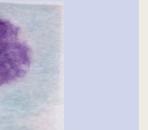
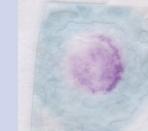
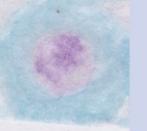
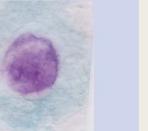
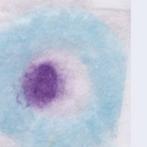
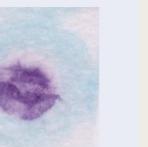
	Treatment Groups	# of Cells	volume of collagen(mircrolitres)	amount of MTT	Notes	Spreading	# of 4-squares
	1	100000	2.00	std			1
	2	125000	2.50	std			1
	3	150000	3.00	std			1
(+ve control	4	125000	2.5	0	no MTT		1
(+ve control	5	125000	2.5	std(left longer)	max MTT	Spread evenly	1
(-ve control	6	250000	5	std			1
	7	100000	2.00	std			1
	8	125000	2.50	std			1
	9	150000	3.00	std			1
(+ve control	10	125000	2.5	0	no MTT		1
(+ve control	11	125000	2.5	std(left longer)	max MTT		1
(-ve control	12	250000	5	std	no spreading		1
		1750000					
	total (including extra):	2275000	45.5				

*Treatment group 10,11,12 are not necessary and eliminated during the experiment

After 24 hours in the incubator, we add MTT for each treatment (50 µl for STD, 100 µl for max)

Result

Figure E1. Cropped images of each sample after 2 hours in the media

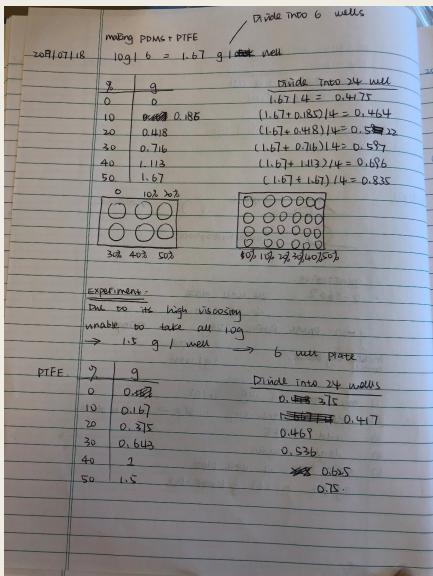
Parameter	A No cell Std MTT	B 2.5 μ L Gel Max MTT	C 5 μ L Gel Std MTT
Control			
	A 2 μ L Gel Std MTT	B 2.5 μ L Gel Std MTT	C 3 μ L Gel Std MTT
Evenly Spread			
Unevenly Spread			

According to Figure E1, the control has indicated that no cells with standard MTT has no color, where average amount of gel with max MTT indicate dark purple. The sample with 5 μ L has shown dark purple, which might be inappropriate amount. Evenly Spread with 3 μ L of gel indicate a decent purple, where all with unevenly indicate darker purple. Therefore, 3 μ L is optimal as a result of this experiment.

F: Other random experiment

- Cell passaging
- Counting cell
- PTFE-PDMS hydrophobic coatings

Figure F1. Calculation for PTFE



*Note during the experiment, the amount has been slightly adjusted due to difficulty in pipetting PDMS.