M1D2: Design experiment to optimize cell loading variables

09/13/18

1. Pre-lab Discussion
2. Instructor Check-in: design parameters
3. Load CometChips: ½ go to TC to prep cells
4. ½ in main lab research the M059J and M059K cell lines

Office Hours

Noreen
Monday 2pm-5pm
in 16-317

Leslie
Thursday 2-3pm
Friday 12-1pm
in 56-341c

Josephine
Wednesday 12-1pm
Friday 2-3pm

Announcements

*Next time meet in 56-614 at 1:05pm for Comm Lab workshop (bring a copy your figure HW)

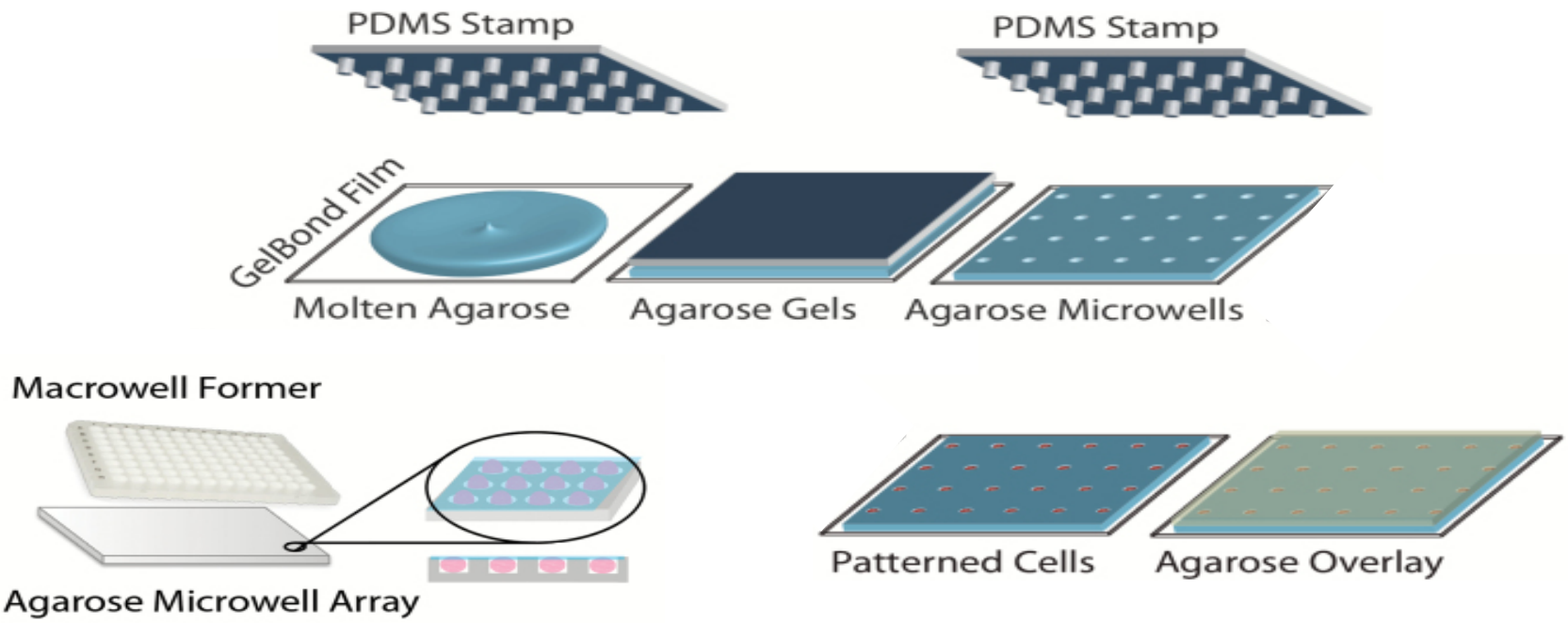
*Remember to spray & wipe benches with 70% ethanol before and after work

M1 major assignments—updated

*Notebooks will be graded at 10 pm the day following the final day of each module (i.e. at 10 pm on the day after M1D7, M2D8, and Research proposal presentations).

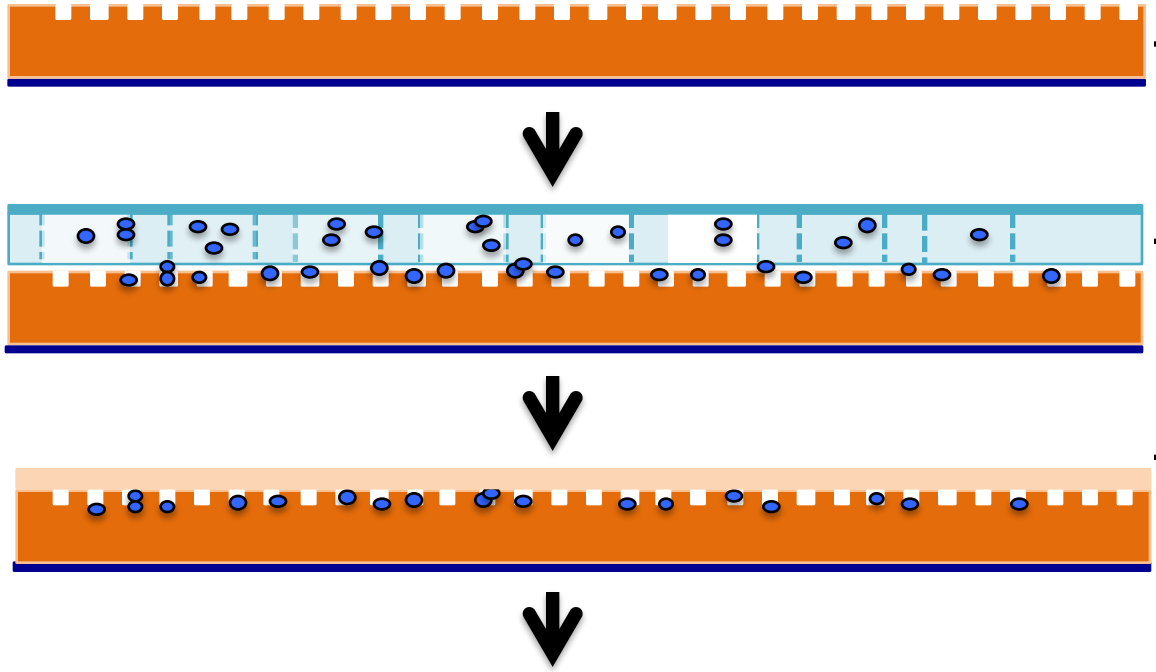
- Data summary (15%)
  - In teams, submit on Stellar
  - Draft due 10/8, final revision due 10/20
  - Bullet points, .PPTX
- Mini-presentation (5%)
  - Individual, submit video via Gmail
  - Due 10/13
- Lab quizzes –be on time!
  - M1D4 and M1D7
- Notebook* (part of 10% Homework and Notebook)
  - Due **10/5** at 10pm, graded by Corban
- Blog: https://be20109f18.blogspot.com (part of 5% Participation)
  - by 10/9

This week: Create a CometChip & optimize cell loading



What is the minimum number of cells needed in each macrowell to obtain efficient loading?

Today: Load cells onto the CometChip



CometChip

Bottomless
96 well plate

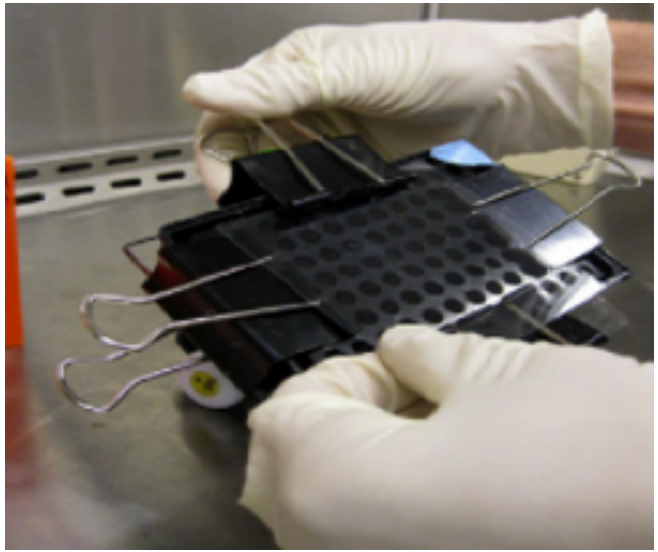
1% LMP
agarose

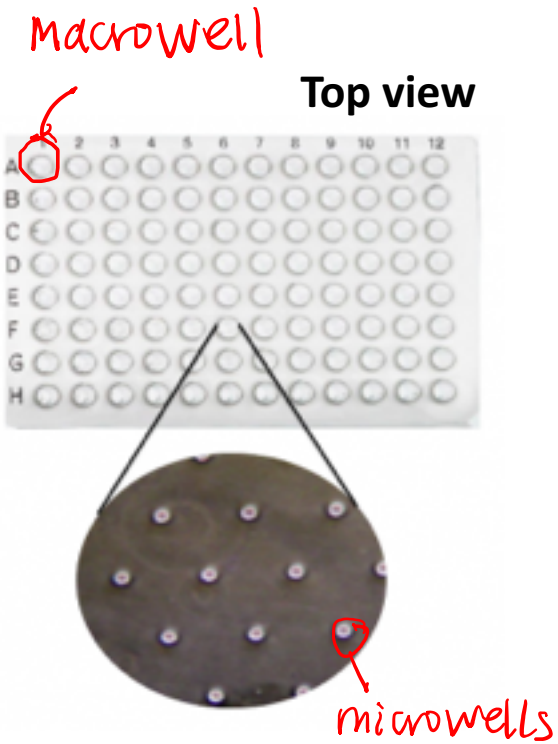
Image on microscope (10X)

side view

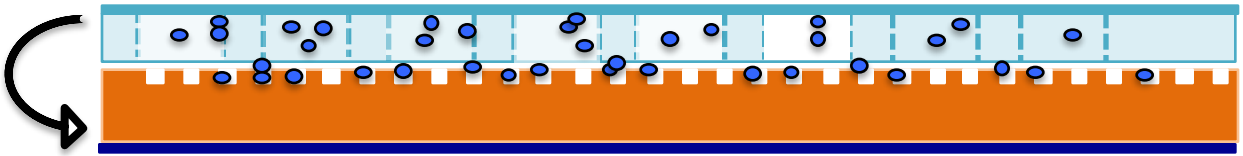
What this looks like in real life

- Glass plate
- Bottomless 96-well plate
- 4 binder clips
- 37°C incubator





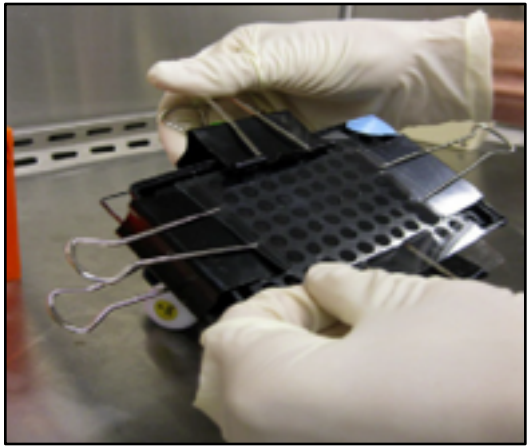
Clamp with binder clips

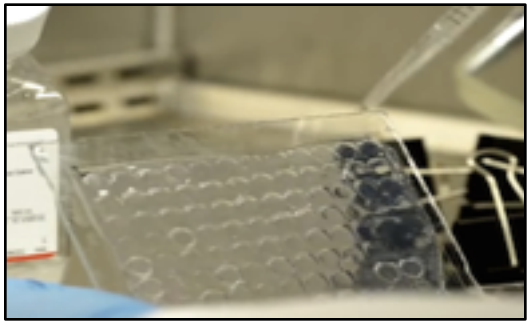


Side view

Critical steps:

- Cell loading
  - Line up macrowells carefully within the pattern drawn on gel bond
- Washing
  - Not too much!
  - Across the top of the glass plate
  - Wash from __low__ to __high__ concentration
  - Don't mix cell types!
- 1% LMP agarose gels *quickly*
  - Leave glass plate under comet chip
  - Dispense it drop-by-drop with P1000
  - Leave it undisturbed for 3 min then move to 4°C for 3 min





Designing the cell loading experiment

<u>Experimental question</u>: What is the minimum number of cells needed in each macrowell to obtain efficient loading?

<u>Considerations:</u>

- size of well (40 μm), distance between wells
- size of cells (~20 μm)

<u>Variable:</u> # cells loaded / macrowell

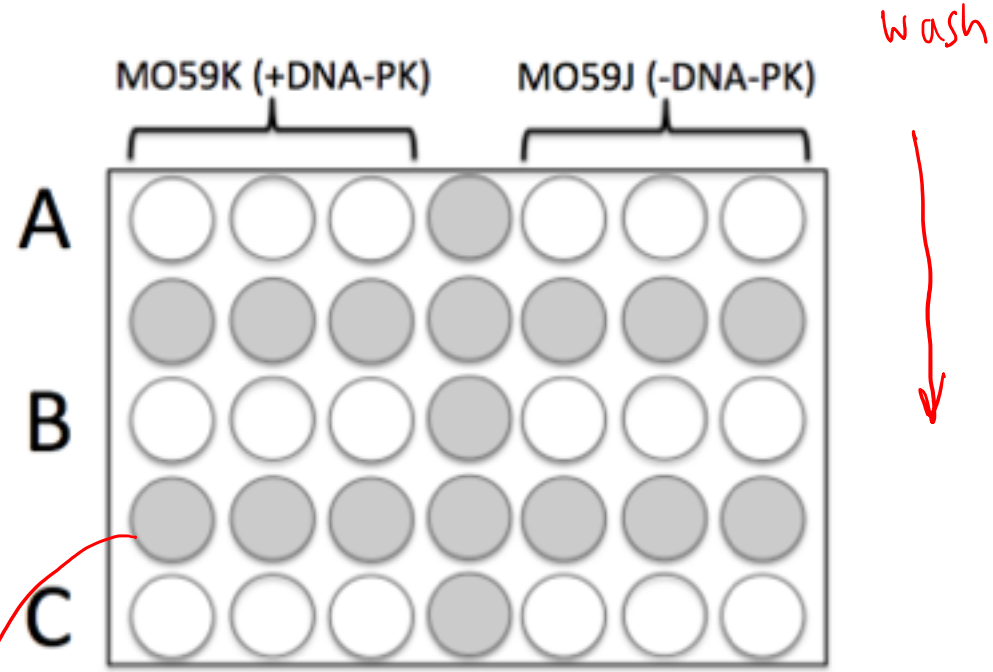
<u>Control:</u> know outcome

neg. control = no cells

<u>Repeatability:</u> replicates (triplicate = 3 macrowells / condition)

Designing the cell loading experiment

A	Condition A will be 'no cells loaded' control
B	Condition B will be the lower cell number decided by your team
C	Condition C will be the higher cell number decided by your team

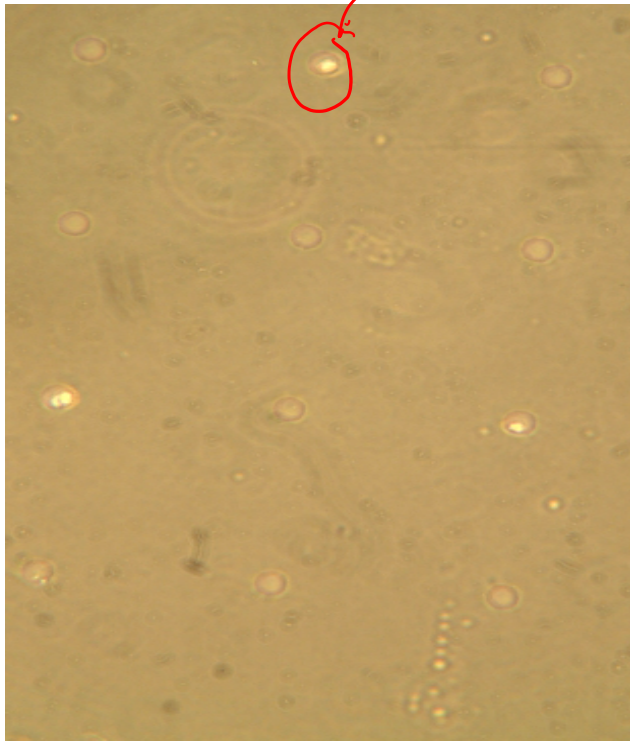


Incubate cells at 37°C for **15min**

Shaded = no cells

Homework and analysis due M1D3

- Make a figure & caption
  - You will receive light microscope images today for your experimental conditions
  - All figures **must include a title and a caption**.
  - Title: Take away message
  - Caption: Info. necessary to describe image/data



- Receive homework credit for visiting Comm. Lab before M1D5!
- Which loading parameters are ideal?
  - Row B or Row C? Keep this info in your lab notebook. We'll discuss next time.

Today in lab:

1. Carefully consider your design parameters and check with an instructor before starting your experiments.
2. ~~Blue and Purple~~ teams to the tissue culture room first to prepare cells
3. ~~Red, Orange, and Green teams start Part 4~~
4. Make sure to get .jpeg images from loading experiment before you leave!

300 microwells/macrowell

microwells ~250μm apart