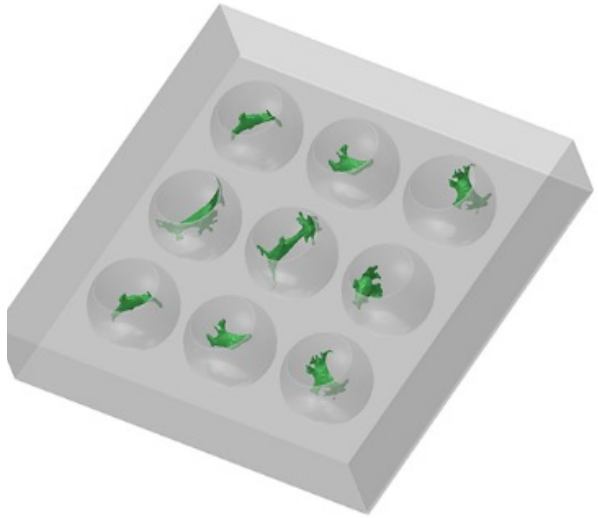
Spherical Microwell Arrays for Mesenchymal Stem Cell Cultures

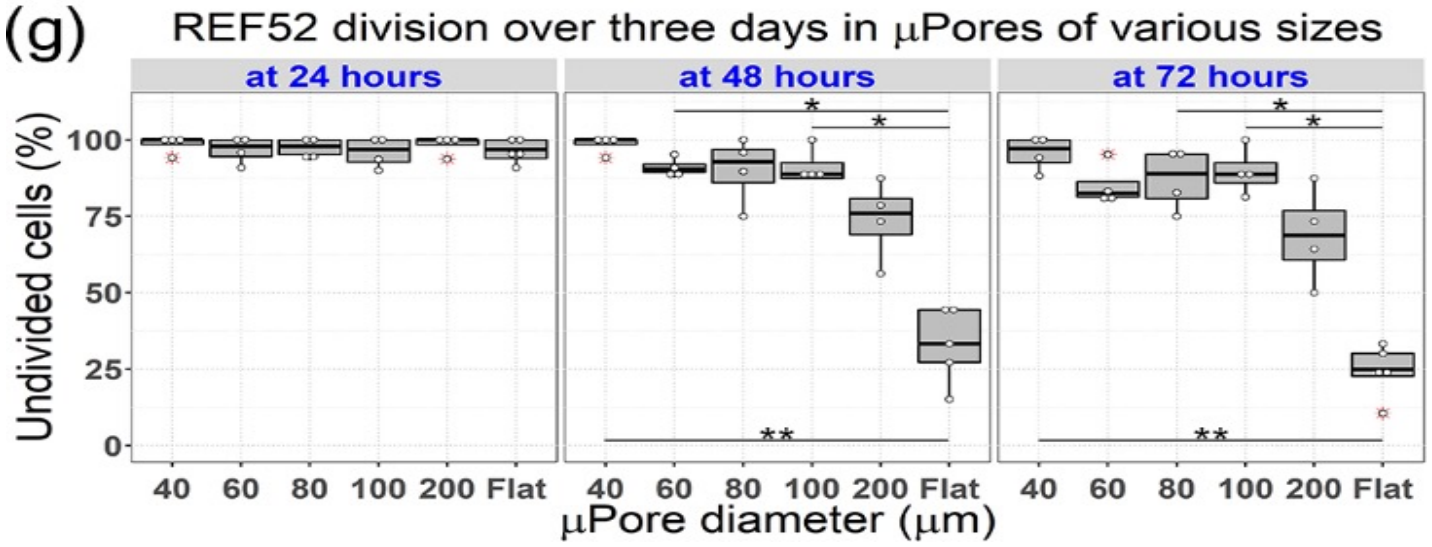


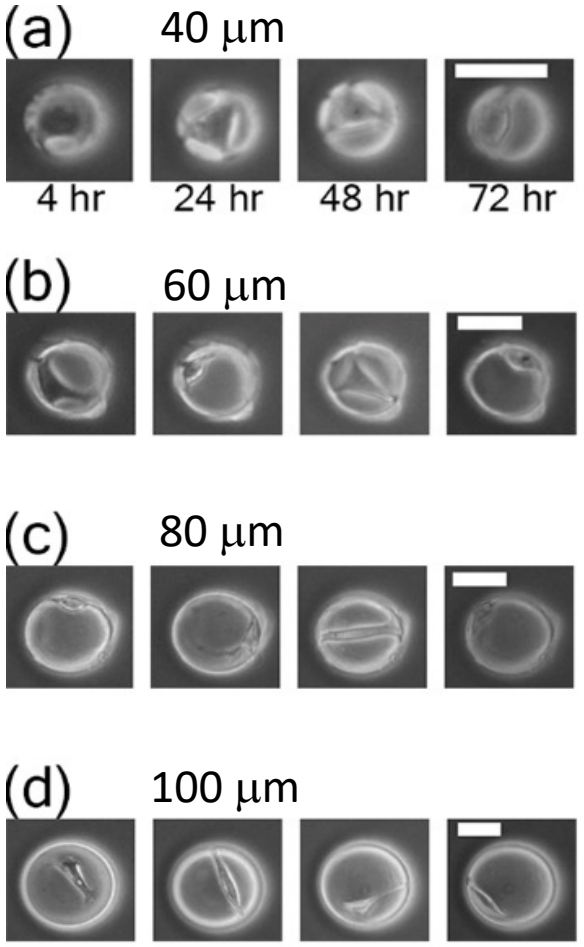
KUO, Hsu-Ting Institute of physics
Supervisor: Dr. Keng-hui Lin

Motivation

In Lin's lab, the former postdoc, Jonny Huang, developed a spherical microwell arrays as a novel 3D culture method.

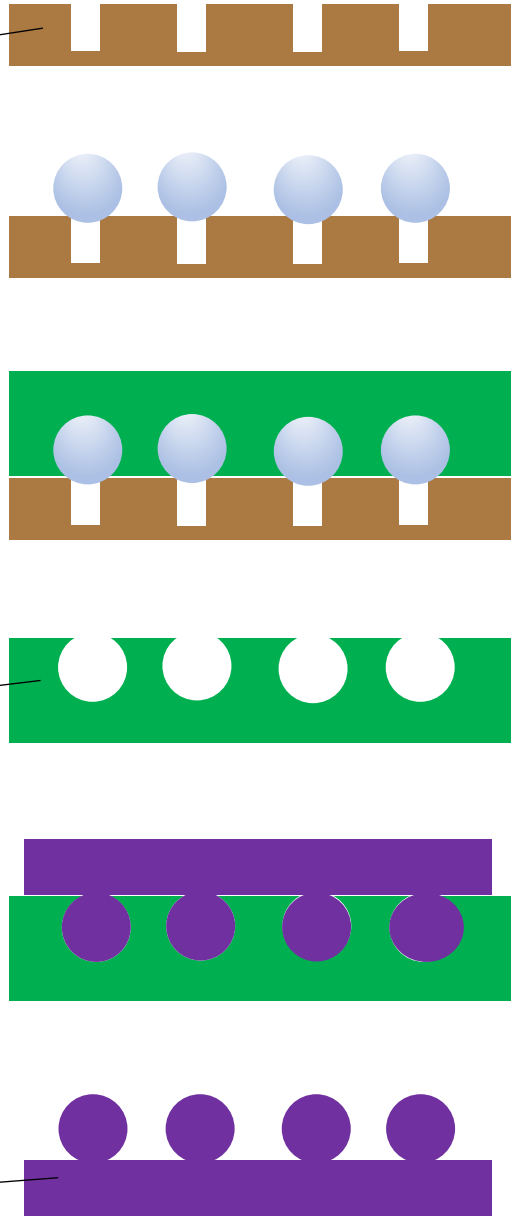
Data shows that the self-renewal ability of REF52 cell decreases under 3D confinement





Rat embryonic fibroblast REF52

Original protocol



Step 1: Create an array of micrometric cylindrical holes on PDMS

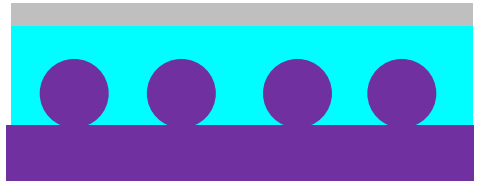
Step 2: Manually placed glass microspheres one by one onto the cylindrical holes

Step 3: Pour fast curing silicone on PDMS

Step 4: Peel off the microspheres

Step 5: The second silicone template is applied with an off-the-shelf two-part epoxy resin and evenly appended to a glass-slide

→ **Step 6**: Obtain monolithic epoxy microsphere mold

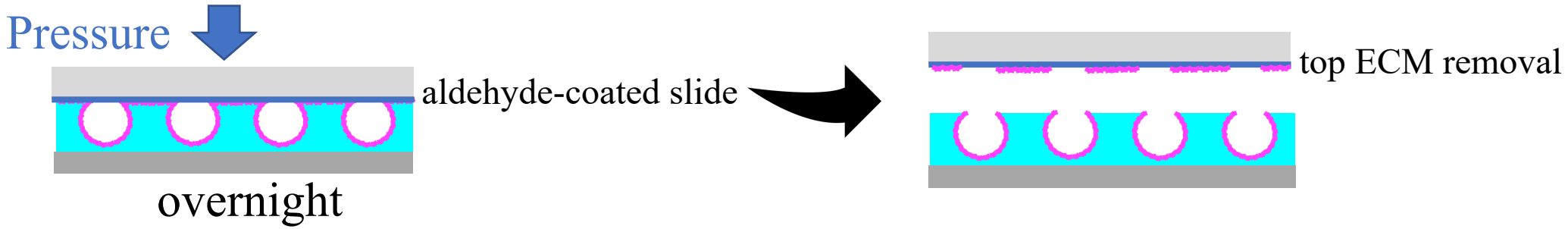


Step 7: Using the mold to make PA gel

Step 8: Sterilization process (UV light)

Step 9: Protein coating on the PA gel

Step 10: Peal off extra protein



Drawback of the Original Protocol

TIME CONSUMING:

AROUND 3 DAYS

10 STEPS

3 DIFFERENT MOLDS INVOLVED

LABOR CONSUMING

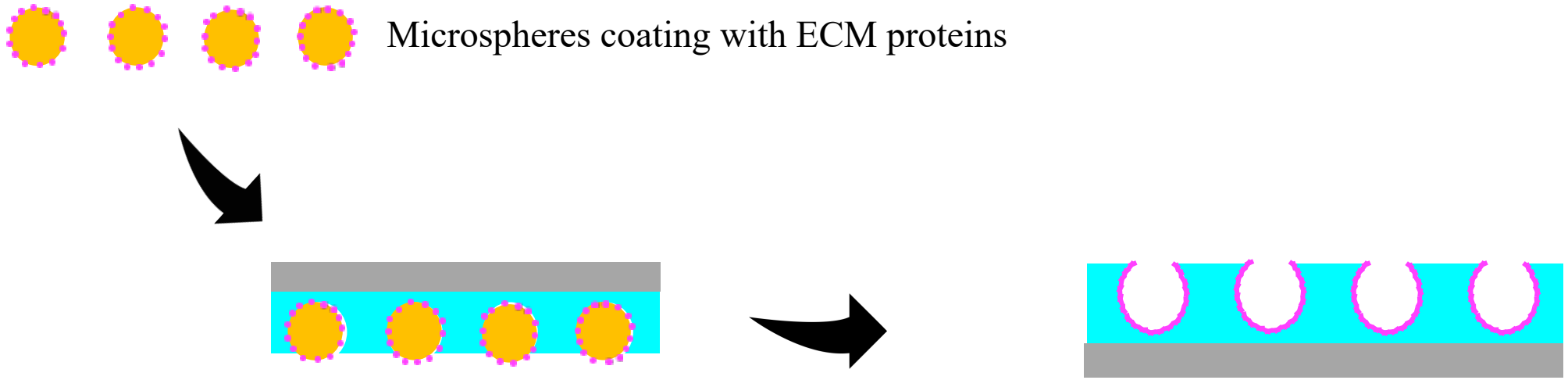
Objective

Our goal is to:

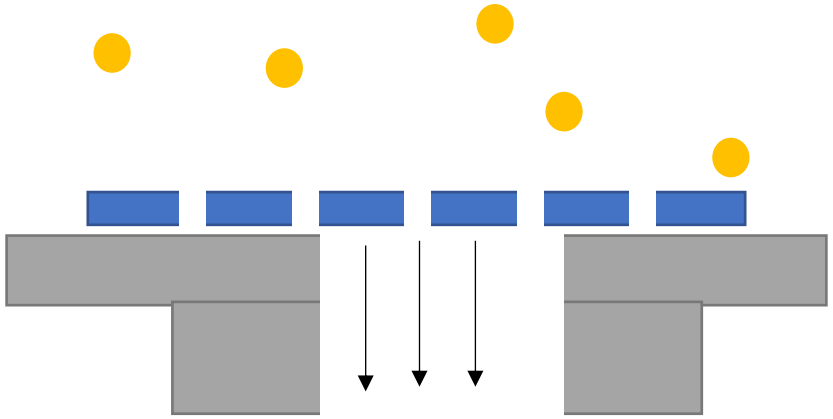
- **Simplify** the protocol of making 3D cell cultured platform
- Observe the change in self-renewal ability of **mesenchymal stem cells (MSC)** under 3D confinement.

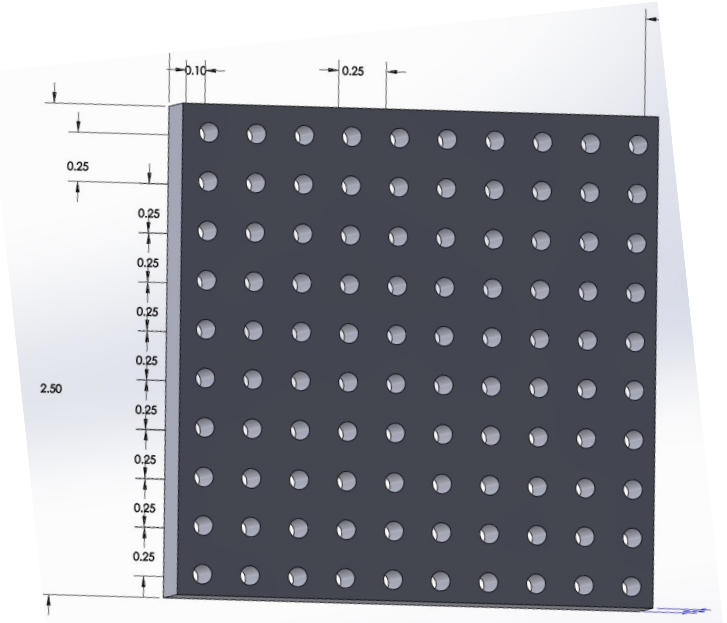
➢ Simplifying steps of making the 3D cell cultured platform

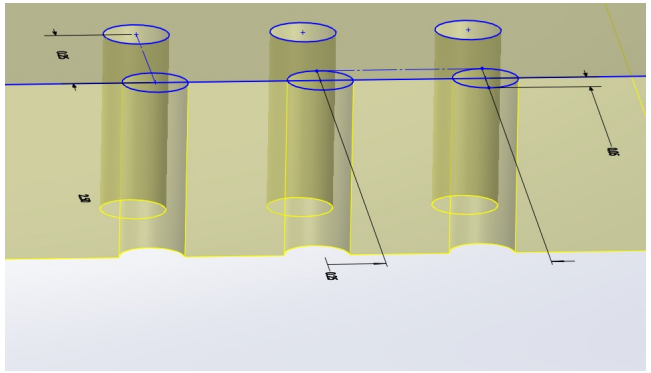
1. Simplify the procedure of beads placing
2. Avoid the step of pealing off extra protein



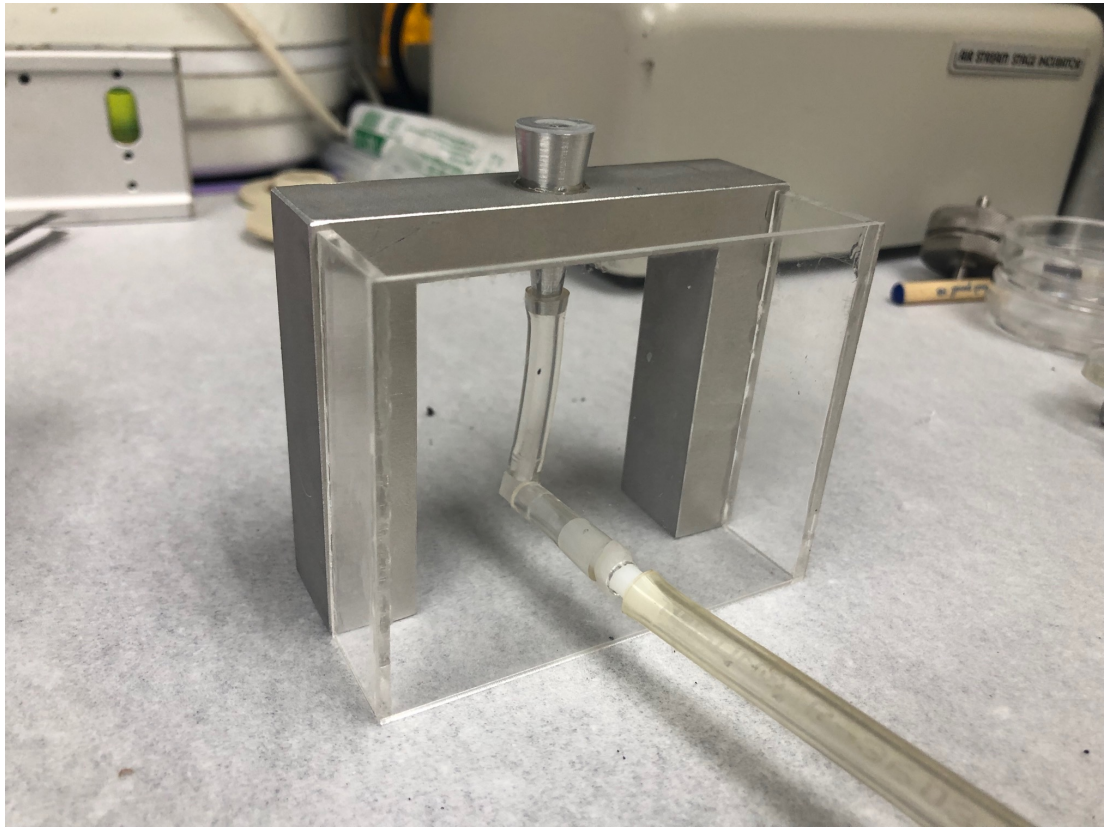
Placing beads on an array of through holes





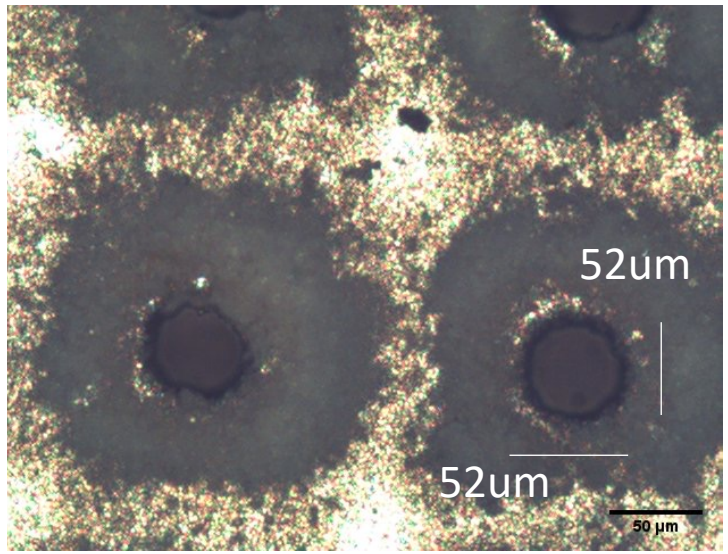


To achieve this purpose, I designed a chuck to hold the hole arrays.

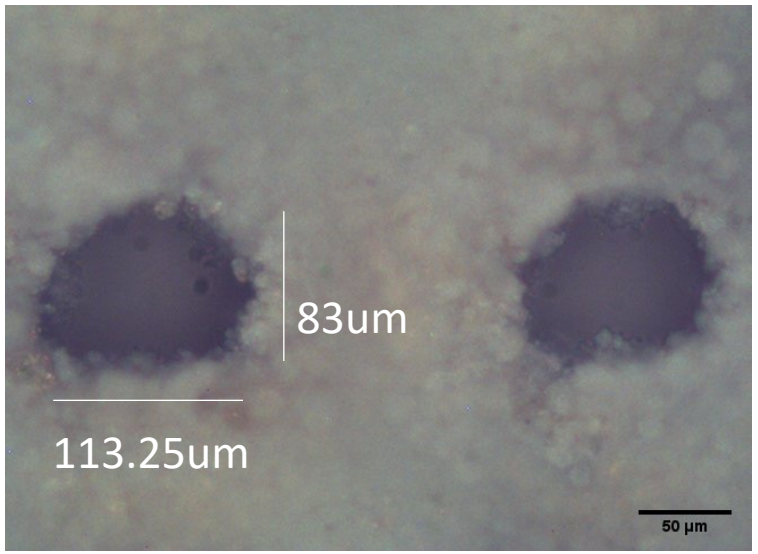


Making Hole Arrays

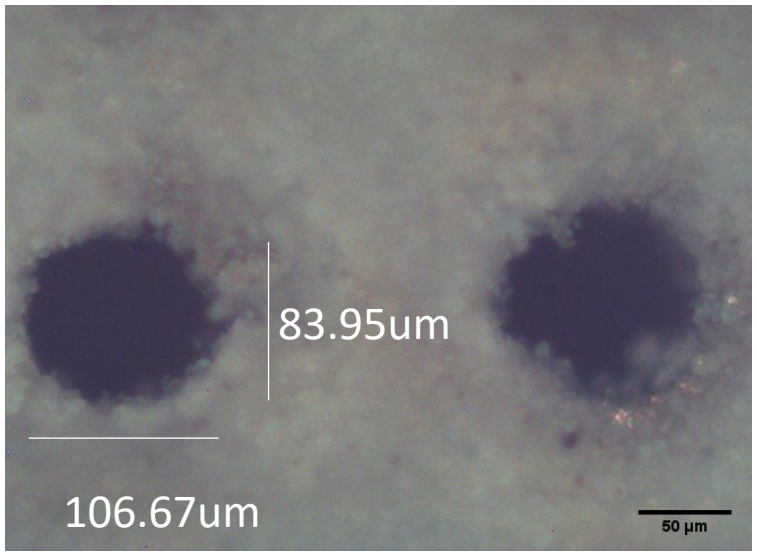
Custom-made by laser hole drilling.



2’’ si wafer
Thickness: 275um



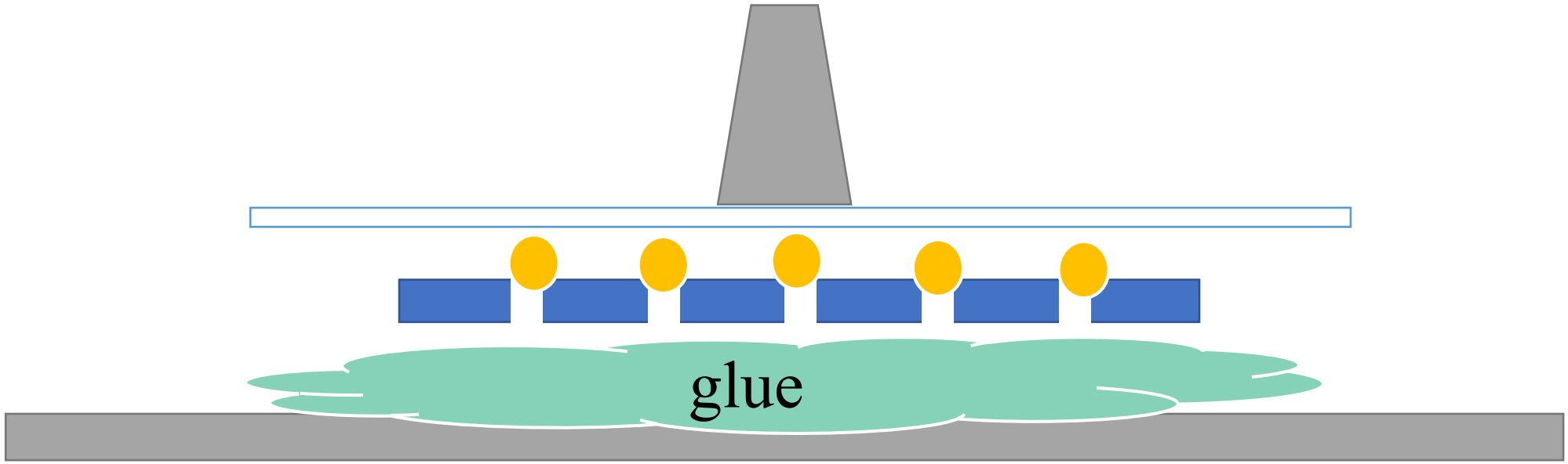
4’’ si wafer
Thickness: 400um



5’’ si wafer
Thickness: 500um

Problem: Non-spherical holes. The vacuum leaks when the spherical spheres sit on top.

Solution:
Use glue to keep beads in place



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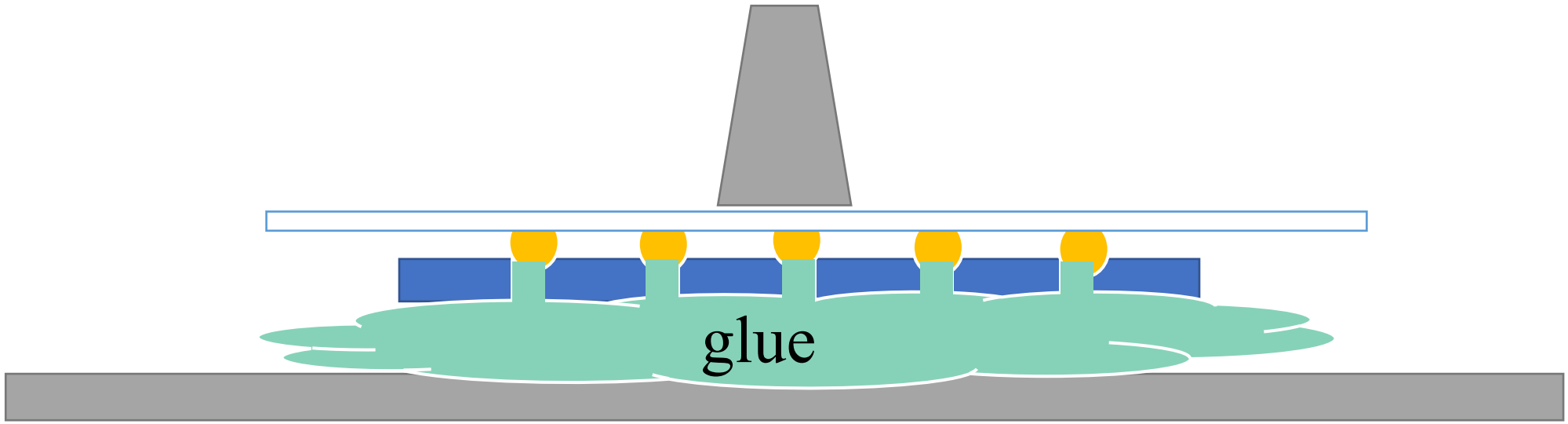
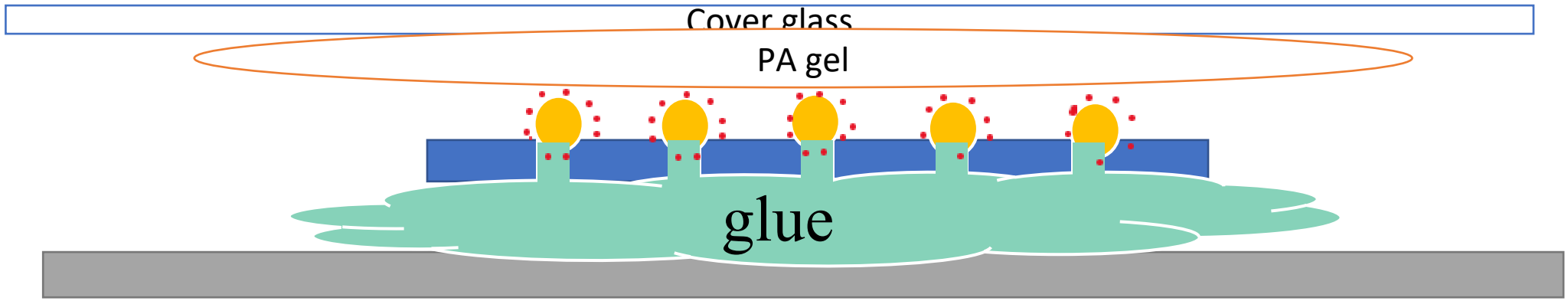


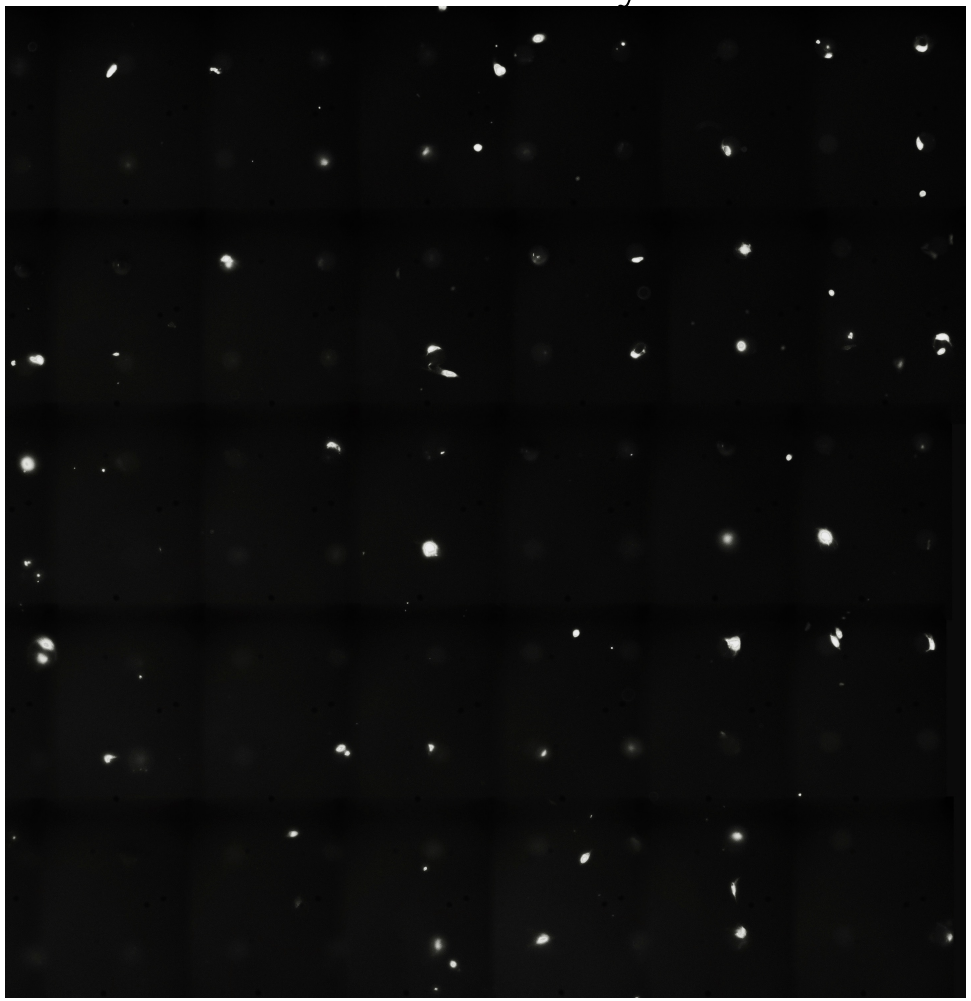
➢ Avoid the step of pealing off protein

1. Coat protein on beads beforehand



MSC grown in 60 mm pores

2019/08/15 Day0

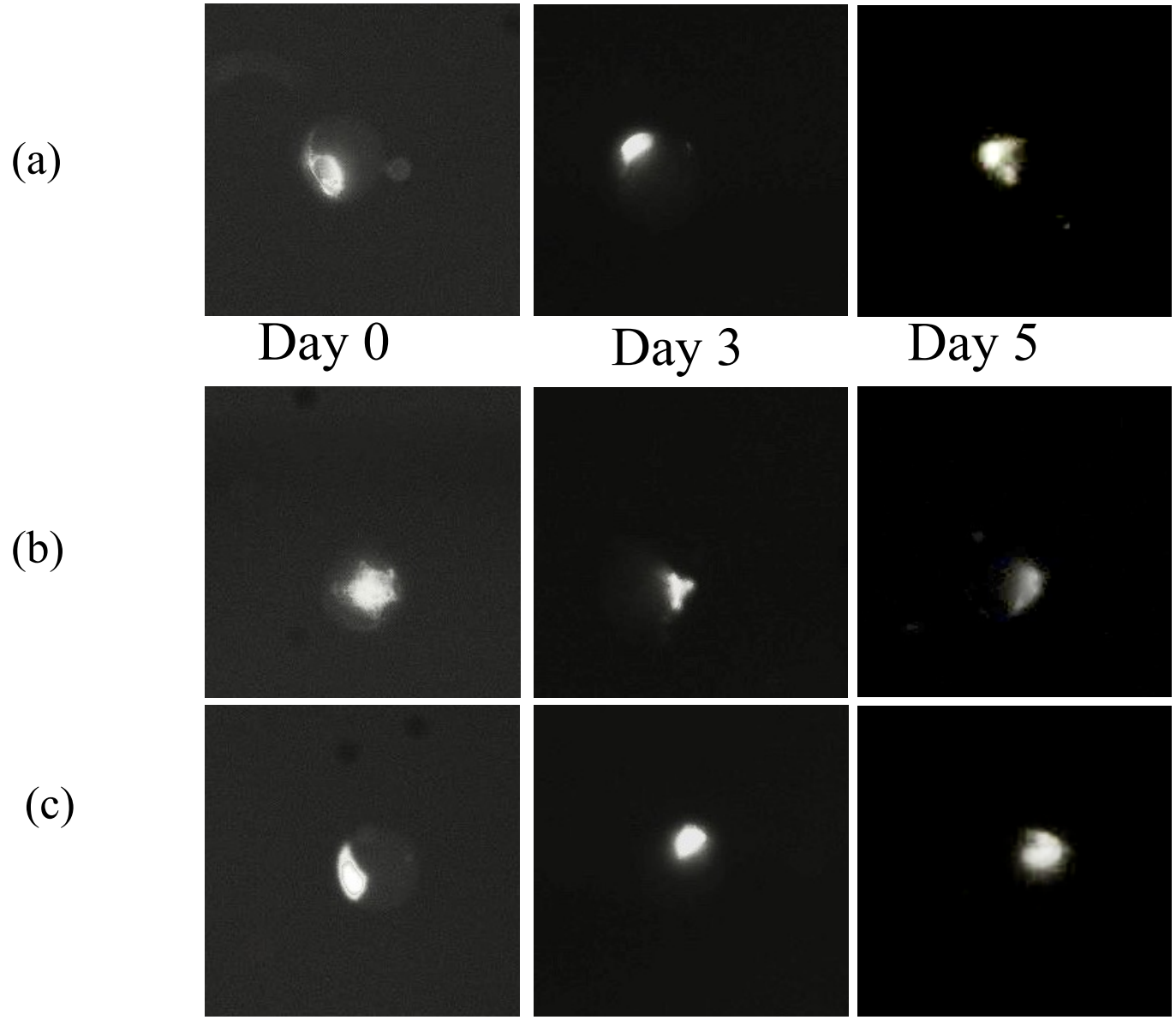


2019/08/19 Day3

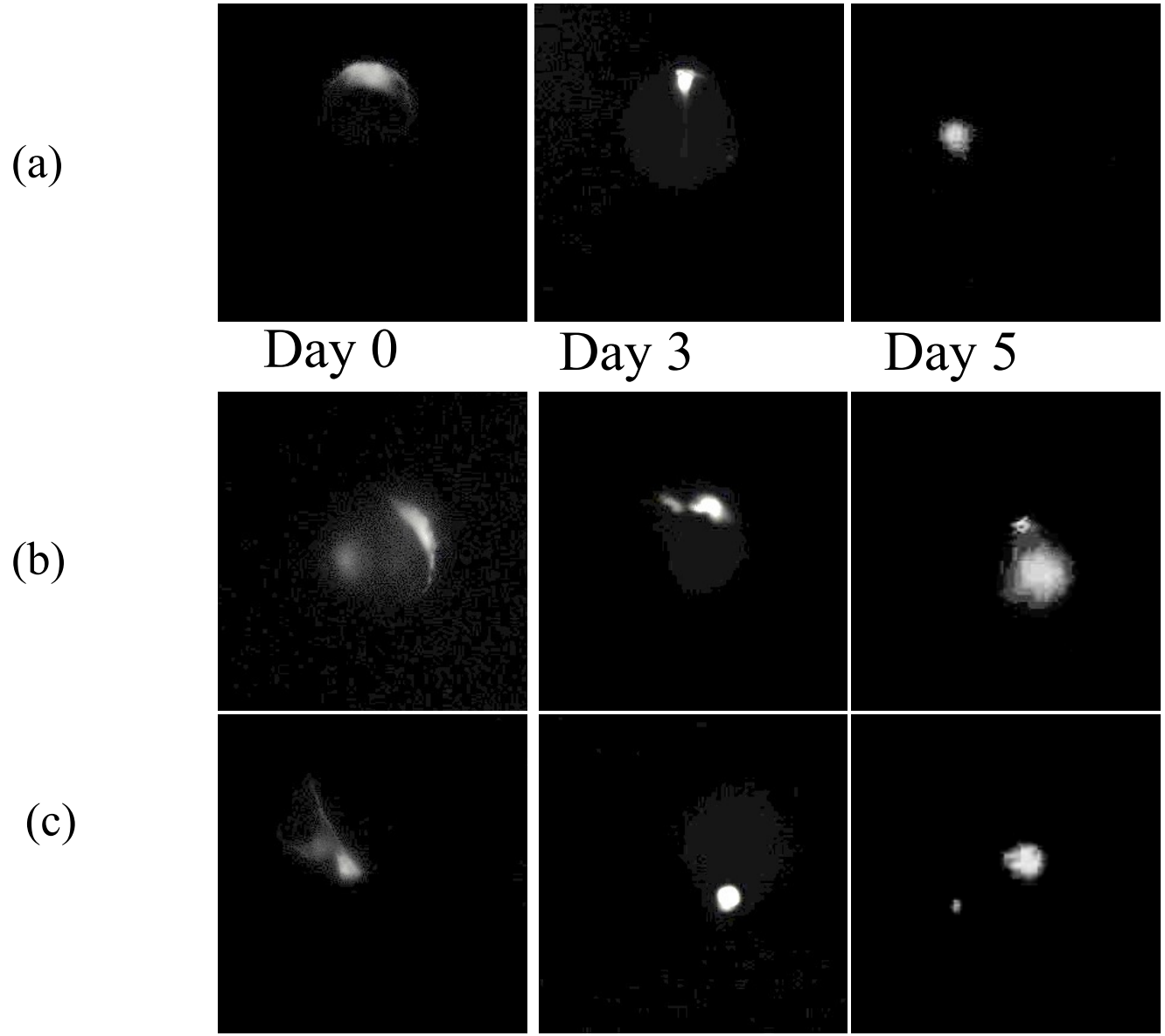
Single cell in pores undivided :
N: 38 out of 38 → 100%

20x TRITC Fluorescent dye: CellTracker

MSC grown in 60 mm pores



MSC grown in 100 μm pores



Conclusion

It is possible to use the new protocol to replace the old method.

We also observe the cell cycle arrest of mesenchymal stem cells in spherical microwell arrays.

Questions?

Thank you!