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Title: Simple, Affordable, and Modular Patterning of Cells using DNA

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   Images but not movies

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Zeiss Axio Vert.A1

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 23
Number of Shots: 57

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Katie Cabral:** CMO-DPAC allows you to pattern cells with very high precision and culture them in 2D or 3D. This opens up opportunities for studies of cell-cell interactions and the morphogenesis of engineered tissues.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Katie Cabral:** The main advantage of this technique is that it is easy for other labs to adopt – it doesn't require a clean room, specialized equipment, or custom-synthesized reagents.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Photopattern DNA onto aldehyde-functionalized slides

2.1. Begin by dropping small drops of the positive photoresist onto the aldehyde slide using a disposable pipette **[1]**. Spin the slide at 3000 rpm for 30 seconds using the spin coater **[2]**, then place it on a 100-degree Celsius hotplate for 1.5 minutes to crosslink the photoresist **[3]**. *Videographer: This step is important!*

2.1.1. Talent dropping the positive photoresist onto the slide.

2.1.2. Talent spinning the slide in spin coater.

2.1.3. Talent placing the slide on hotplate for soft bake.

2.2. Remove the slide from the hotplate and place a photomask with the desired features on top of the slide **[1]**. Weigh it down with a piece of glass and cover the entire setup in an opaque box **[2-added]**.

2.2.1. Talent placing photomask on top of the slide.

2.2.2. Added shot: Talent placing glass disc on slide, then covering it with an opaque box.

2.3. Expose the setup with a UV lamp for 2 minutes **[1-TXT]**. Develop the slide by immersing it in the developer solution for 3 to 5 minutes **[2]**, then rinse away excess developer solution with water **[3]**. Dry it under a stream of air or nitrogen **[4]**.

2.3.1. Talent exposing the setup to UV lamp. **TEXT: 365 nm wavelength, 360 mW, 5 inches from slide, total radiant energy density 100 mJ/cm^2**

2.3.2. Talent immersing the slide in developer solution.

2.3.3. Talent rinsing the slide with water.

2.3.4. Talent drying the slide under air or nitrogen.

2.4. Observe the slide under a microscope to confirm the success of photolithography and store it in the dark **[1]**.

2.4.1. Talent observing the slide under microscope.

2.5. Add a droplet of 20 micromolar amine-modified oligo solution onto each photopatterned region of the slide **[1]** and spread the droplet gently across the entire region using a pipette tip, taking care to not scratch the slide **[2]**.

2.5.1. Talent adding drop of amine-oligo solution.

2.5.2. Talent spreading the droplet with a pipette tip.

2.6. Bake the slide in a 65-degree Celsius oven until the DNA solution has fully dried **[1]**. Perform reductive amination by placing the baked slide in a 15-centimeter cell-culture dish in a fume hood on top of a shaker **[2]**.

2.6.1. Talent placing the slides in oven.

2.6.2. Talent placing the dish containing baked slides on a shaker kept in a fume hood.

2.7. Gently mix 100 milligrams of sodium borohydride in 40 milliliters of PBS and add it to the dish, then turn on the shaker for 15 minutes **[1]**.

2.7.1. Talent adding the sodium borohydride solution to the dish and turning on the shaker.

2.8. After the reaction, wash the slide twice with 0.1% sodium dodecyl sulfate to remove unreacted DNA, then wash the slide three times with water **[1]**. Dry the slide under the stream of nitrogen or air **[2]**. Finally, rinse it with acetone to remove the remaining photoresist **[3]**.

2.8.1. Talent washing the slide with SDS.

2.8.2. Talent drying the slide under air or nitrogen.

2.8.3. Talent rinsing the slide with acetone.

3. Lift and label cells with cholesterol-modified DNA

3.1. Prepare a 4 micromolar universal anchor and adapter solution as described in the text manuscript **[1]** and a 20 micromolar universal co-anchor solution in PBS **[2]**.

3.1.1. Prepared universal anchor and adapter solution, with the tube labeled.

3.1.2. Talent adding the solution mixture to PBS.

3.2. Prepare the cell suspension by resuspending the cell pellet in 1 milliliter of ice-cold PBS or serum-free media **[1]** and transfer 1 to 3 million cells to a 1.5-milliliter microcentrifuge tube **[2]**. Centrifuge at 160 times *g* for 4 minutes **[3]**.

3.2.1. Talent resuspending the cells in PBS or serum-free medium.

3.2.2. Talent transferring the cells to a microcentrifuge tube.

3.2.3. Talent centrifuging the tube.

3.3. Resuspend the obtained cell pellet in 75 microliters of ice-cold PBS and serum-free media **[1]** and add 75 microliters of the prepared 4 micromolar universal anchor and adapter solution **[2]**. Mix thoroughly and incubate for 5 minutes on ice **[3]**.

3.3.1. Talent adding PBS to cell pellet.

3.3.2. Talent adding prepared universal anchor and adapter solution.

3.3.3. Talent putting the tube on ice.

3.4. Add 15 microliters of the Universal Co-anchor solution to the tube and mix thoroughly, then incubate the sample for 5 minutes on ice **[1]**.

3.4.1. Talent adding Universal Co-anchor solution to the tube and mixing it.

3.5. To remove excess of oligos from the cell suspension, add 1 milliliter of ice-cold PBS or serum-free media to the tube and mix with the pipette **[1]**. Pellet the cells by centrifuging at 160 times *g* for 4 minutes at 4 degrees Celsius **[2]**, then discard the supernatant **[3]**.

3.5.1. Talent adding PBS or serum-free media to the tube and mixing.

3.5.2. Talent centrifuging the tube.

3.5.3. Talent discarding the supernatant

4. Pattern the DNA-labeled cells and transferring into hydrogel for 3D culture

4.1. Resuspend the cells in ice-cold PBS or serum-free media to create a cell-dense solution of at least 25 million cells per milliliter **[1]**. Slightly tilt the patterned slide, then add 25 microliters of this cell suspension to the inlet of each flow cell **[2]**. *Videographer: This step is important!*

4.1.1. Talent resuspending the cells in PBS.

4.1.2. Talent adding cell suspension on the patterned slide.

4.2. Remove the PBS and 1% BSA solution from the outlet, allowing the cell suspension to fill the PDMS flow cell **[1]**. Incubate on ice or at room temperature for 30 seconds **[2]**. *Videographer: This step is important!*

4.2.1. Talent removing PBS and BSA solution for the outlet of the slide.

4.2.2. Talent incubating the slide on ice or RT.

4.3. Aspirate 5 microliters of the cell suspension from the outlet of the slide **[1]** and add it back into the inlet **[2]**. Repeat this 10 times per flow cell **[3]**.

4.3.1. Talent aspirating cell suspension from the slide outlet and adding the cell suspension to the slide inlet. NOTE: 4.3.1 – 4.3.2 together

~~4.3.2. Talent adding the aspirated solution back to the slide.~~

4.3.3. SCOPE: Cells being added to the slide, mixed, and then washed out, revealing the pattern NOTE: Do not use the shots taken during filming, please use the SCOPE shots uploaded to the AWS project page (https://www.jove.com/account/file-uploader?src=18890833). Author didn't like the shots frm the shoot.

4.4. Gently pipette PBS or serum-free media into the inlet of each flow cell to wash out the excess cells **[1]** and collect the cell suspension from the outlet **[2]**. Repeat this 2 to 4 times until no excess cells remain on the slide **[3]**.

4.4.1. Talent pipetting PBS to the inlet of each flow cell.

4.4.2. Talent collecting the cell suspension from the outlet.

4.4.3. SCOPE: Slide with no excess cells. NOTE: Do not use the shots taken during filming, please use the SCOPE shots uploaded to the AWS project page (https://www.jove.com/account/file-uploader?src=18890833). Author didn't like the shots frm the shoot.

4.5. For 3D culture, prepare a hydrogel precursor solution containing 2% DNase and add 50 microliters of it to the inlet of each flow cell **[1]**. Aspirate the excess fluid from the outlet, driving the hydrogel solution into the flow cell **[2]**.

4.5.1. Talent adding the DNase solution to the inlet.

4.5.2. Talent aspirating the excess fluid from the outlet.

4.6. Incubate the slide at 37 degrees Celsius for 30 to 45 minutes to allow the hydrogel to set and to cleave the DNA-based adhesion between the cells and the surface **[1]**.

4.6.1. Talent incubating the slide at 37-degree Celsius.

4.7. Add 50 microliters of hydrogel precursor to a well of a 2-well chamber slide or a 6-well plate **[1]**. Pipette 10 microliters of PBS on either side of each flow cell **[2]**. Distribute it along the full length of the flow cell using a razor blade or fine-point tweezers and gently lift the sides of the flow cell so that the PBS rushes under the hydrogel **[3]**. *Videographer: This step is important!*

4.7.1. Talent adding hydrogel precursor to the plate wells.

4.7.2. Talent adding PBS to on either side of each flow cell.

4.7.3. Talent distributing the PBS along the flow cell and lifting the sides of the flow cell.

4.8. Using a razor blade, move the flow cell to the edge of the slide by inverting the slide **[1]** and nudge the flow cell off the slide so that it lands on top of the razor blade **[2]**. Pick the flow cell off the razor blade using curved forceps very slowly **[3]**. *Videographer: This step is difficult and important!*

4.8.1. Talent moving the flow cell to the edge of the slide, inverting the slide, and then nudging the flow cell onto the razor blade. NOTE: 4.8.1 – 4.8.2 combined

~~4.8.2. Talent nudging the flow cell from the slide onto the razor blade.~~

4.8.3. Talent picking the flow cell using forceps.

4.9. Invert the flow cells so that the cells are on the bottom and place them on top of the droplet of hydrogel precursor solution **[1]**. Incubate for at least 30 minutes so that the hydrogel containing the patterned cells can bind to the hydrogel underlay, resulting in the full embedding of the patterned cells **[2]**. *Videographer: This step is important!*

4.9.1. Talent inverting the flow cells and placing on top of the droplet.

4.9.2. Talent incubating the flow cell.

4.10. Remove the flow cell and immerse it completely in media **[1]**. Gently nudge the flow cell using curved forceps until it pops off and floats into the media, then discard it **[2]**. *Videographer: This step is important!*

4.10.1. Talent immersing the flow cell in media.

4.10.2. Talent nudging the flow cell in the media and then discarding it.

Results

5. Adhesion of CMO-labeled cells to DNA patterns

5.1. The quantification of the DNA spot adhesion to CMO-labeled cells, which increases as a function of CMO concentration, is represented as the mean standard deviation from three experiments **[1-TXT]**. The DNA patterns are shown in magenta **[2]** and the adhered CMO-labeled cells in cyan at different concentrations of CMO **[3]**.

5.1.1. LAB MEDIA: Figure 4A. **TEXT: CMO-Cholesterol modified oligos**

5.1.2. LAB MEDIA: Figure 4B. *Video Editor: Emphasize the magenta dots.*

5.1.3. LAB MEDIA: Figure 4B. *Video Editor: Emphasize the cyan dots.*

5.2. A comparison of CMO-labeled HUVECs and LMO-labeled HUVECs adhered to a linear DNA pattern is shown here **[1-TXT]**.

5.2.1. LAB MEDIA: Figure 5A. **TEXT: HUVECs-human umbilical vein endothelial cells, LMO-lipid modified oligos**

5.3. Single MDCKs patterned via CMO-DPAC and transferred into Matrigel were able to proliferate and polarize after 5 days of culture **[1]**. Multilayered, multicellular aggregates were created by alternating layers of cells labeled with complementary CMOs **[2]**.

5.3.1. LAB MEDIA: Figure 5B. **TEXT: MDCKs- Madin-Darby Canine Kidney cells, DPAC-DNA programmed assembly of cells**

5.3.2. LAB MEDIA: Figure 5C.

5.4. Multiple unique cell populations can be patterned together with high precision and without cross-contamination **[1]**.

5.4.1. LAB MEDIA: Figure 6.

Conclusion

6. Conclusion Interview Statements

6.1. **Katie Cabral:** When attempting this protocol, it is critical to have a dense cell suspension while adding cells to the slide in order to maximize the opportunities for cells to stick to a DNA spot.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1*