



MDCK acini formation protocol on CYTOOchips™

Creation date :

15/05/13

MO-EXT-20

A- Objective

Describe how to form well organized polarized acini from MDCK cells on CYTOOchips (CC20).

B- Thawing Matrigel™

Thaw Matrigel on ice overnight at 4°C. To avoid multiple freeze thaws, make little aliquots (50 µl and 100 µl aliquots for example) conserved at -80°C. Please read MO-EXT-19 for our hints on Matrigel manipulation.

C- Coating preactivated CYTOOchips with Laminin or Collagen I

- Put chips in 6 wells plate or in a Petri Dish 35x10mm
- Immerge each chip in 2 ml of PBS (without Ca²⁺/Mg²⁺)
- Prepare a 3x concentrated protein solution at 60 µg/ml in PBS
- Add 1 ml of 3x protein solution on each immersed chip; final concentration = 20 µg/ml
- Incubate 2 hours at Room Temperature or over night at 4°C
- Wash without drying the chip with about 20 - 30 ml of PBS:
Either, wash in a continuous flow: aspirate out protein solution and add in PBS at the same time.
Or, wash with successive dilutions: to the 3 ml of protein solution add 3 ml of PBS, remove 4 ml, add 4 ml PBS, remove 4ml, add 4ml PBS, remove 4ml, add 4 ml PBS etc... at least 6 times.
If you want to seed cells immediately, leave the chip in the last wash of PBS for about 20 minutes before removing all the PBS and then adding the cells. If you want to seed the next day, leave in the last PBS wash over night at 4°C.

D- Seeding MDCK cells

- Take your protein coated chip from the fridge to warm up to room temperature
- Take a confluent flask of MDCK cells (60-80% confluent) and wash twice with PBS : the first time quickly and the second time for 30 minutes at RT
- Trypsinize cells for 10 minutes at 37°C
- Resuspend the cells in Growth Medium; possibility to spin the cells at low speed (4000 x g) to completely eliminate trypsin.
- Count cells
- Prepare a cell suspension of 10 000 cells/ml in "acini medium".
For MDCK-NBL2 cells this is the same as the growth medium = MEM + 10% SVF + 1mM Na Pyruvate + 0.5% Pen/Strep; For MDCKII cells the acini medium = MEM + 2% FCS with no Na Pyruvate.
We have found that when cells proliferate too fast they do not form polarized acini correctly. By using 2% FCS and no Na pyruvate growth is slowed down.
- Remove all PBS from the coated chips
- Add 2ml of the cell suspension ie. 20 000 cells total to each chip
- Incubate 30 minutes to 1 hour at room temperature without moving the plate
- Incubate at 37°C in cell incubator for 3-4 hours.

E- Overlay with Matrigel

After 3-4 hours of incubation when cells are adhered to the pattern, we add Matrigel in the medium as an overlay

- Use Matrigel defrosted overnight at 4°C
- Prepare a matrigel solution of 5% in cold "acini medium" to keep the Matrigel in liquid state



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- Remove 1 ml of medium from the chip
- Add 1 ml of the 5% Matrigel solution; final concentration = 2.5%
- Incubate at 37°C for 3 days.

F- Immunofluorescence

All removal or addition of liquid to the chip has to be gentle in order to not lose the acini which are not firmly stuck to the pattern.

- Fixation:
 - Remove the medium and add 2 ml/wells of Formalin or PFA 4% for 20 minutes at room temperature
 - Wash 2 times with PBS; possibility to store in PBS at 4°C some days.
- Permeabilization:
 - Prepare a solution of 0.2% Triton X-100 in PBS
 - Remove PBS and add 1.5 ml/well for 15 minutes at 4°C and wash 1 time with PBS
- Blocking:
 - Prepare a solution of 3% BSA in PBS
 - Remove PBS and add 2ml/wells for 30 minutes at room temperature
- Primary Antibody:
 - Prepare a dilution of your antibody in a solution of 1.5% BSA in PBS.
 - Mouse anti-GP135: *lumen and apical polarity marker* (Keith Mostov Lab¹) 1/500
 - Rabbit anti β -catenin: *adhesion junctions marker* (Invitrogen 71-7200) 1/500
 - Put 150 μ l of antibody on a piece of parafilm and return the chips on the drop
 - Incubate for 1-2 hours at room temperature according to your antibody
 - Return chips on wells and wash 3 times 10 minutes with PBS
- Secondary Antibody:
 - Prepare the dilution of your antibody in a solution of 1.5% BSA.
 - Phalloidin-FITC: *actin marker* (Sigma P5282) 1/200
 - Anti-Mouse 546 (Jackson 715-165-151) 1/400
 - Anti-Rabbit 649 (Jackson 711-495-152) 1/400
 - Put 150 μ l of antibody on a piece of parafilm and return the chips on the drop
 - Incubate for 1 hour at room temperature according to your antibody
 - Return chips on wells and Wash 3 times of 10 minutes with PBS
- Hoechst staining:
 - Prepare a solution of Hoechst (1/20 000) in PBS.
 - Remove PBS and add 2 ml/wells for 3 minutes at room temperature
 - Wash 1 time with PBS
- Mounting:
 - Put a drop of mounting liquid (ex: 35 μ l of Prolong Gold) on a glass slide
 - Rinse chips in a beaker of water and dry chips on a absorbent paper
 - Return chips on the drop
 - Let dry over night at room temperature before conserving at 4°C in the dark

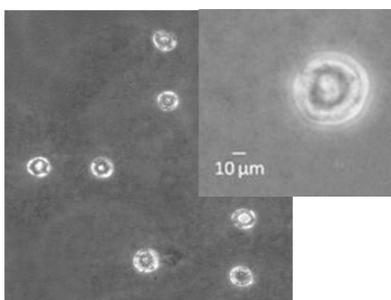
¹ Address for gp135 antibody requests: George Ojakian, Professor at Dept of Anatomy and Cell Biology, State University of New York (SUNY) Downstate Medical Center, Brooklyn, NY. george.ojakian@downstate.edu

G- Table of Reagents

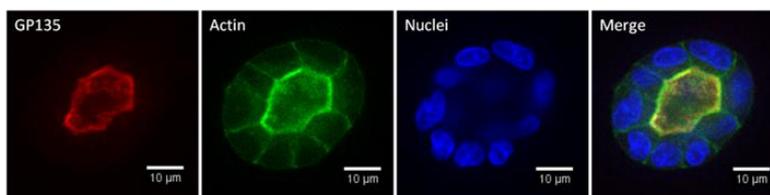
Products	Suppliers	References
Chips Starter's CC20	CYTOO	10-900-X
Chips Disc Small CC20	CYTOO	10-001-X
MDCK-NBL2	ATCC	CCL-34
MDCKII	ECACC	00062107
Matrigel	Becton Dickinson (BD biosciences)	354234
Laminin	Sigma-Aldrich	L2020
Collagen type 1 from calf skin	Sigma-Aldrich	C8919
PBS - (without Ca ²⁺ /Mg ²⁺)	Invitrogen	14190-094
Trypsin	Invitrogen	25300-054
MEM	Invitrogen	41090-028
Foetal Bovine Serum (lot A10107-1008)	PAA	A15-101
Penicillin-Streptomycin	Invitrogen	15140-122
Formalin	Sigma-Aldrich	HT5011-1CS
Triton™ X-100	Sigma-Aldrich	T9284
Albumin from bovine serum	Sigma-Aldrich	A7906
Hoechst	Invitrogen	H3570
Prolong Gold Antifade Reagent	Invitrogen	P36930

H- Typical results

Using the above protocol, we can analyze about 2000 structures and we generally obtain 60-80% of polarized acini by 72 hours after seeding, depending on the micropattern size and shape used. For MDCK, the formation of polarized acini is favoured on the small disc pattern (700 μm^2). You can also leave acini to continue their development beyond 3 days on micropatterns but you will have to change the Matrigel every 3 days. Consult document MO-EXT-19 for how to change the Matrigel without drying out cells.



Phase contrast image of a 3 day old acini taken with a wide field microscope. The lumen is easily visible and can even be seen as early as 24-48 in MDCK cells. Using the CYTOOchips allows an array of acini to be built, with structures all in the same Z plane, greatly facilitating imaging and image analysis.



Single slice confocal image through a micropatterned acinus showing apical marker gp135 antibody, actin stained with phalloidin and nucleus stained with Hoechst.