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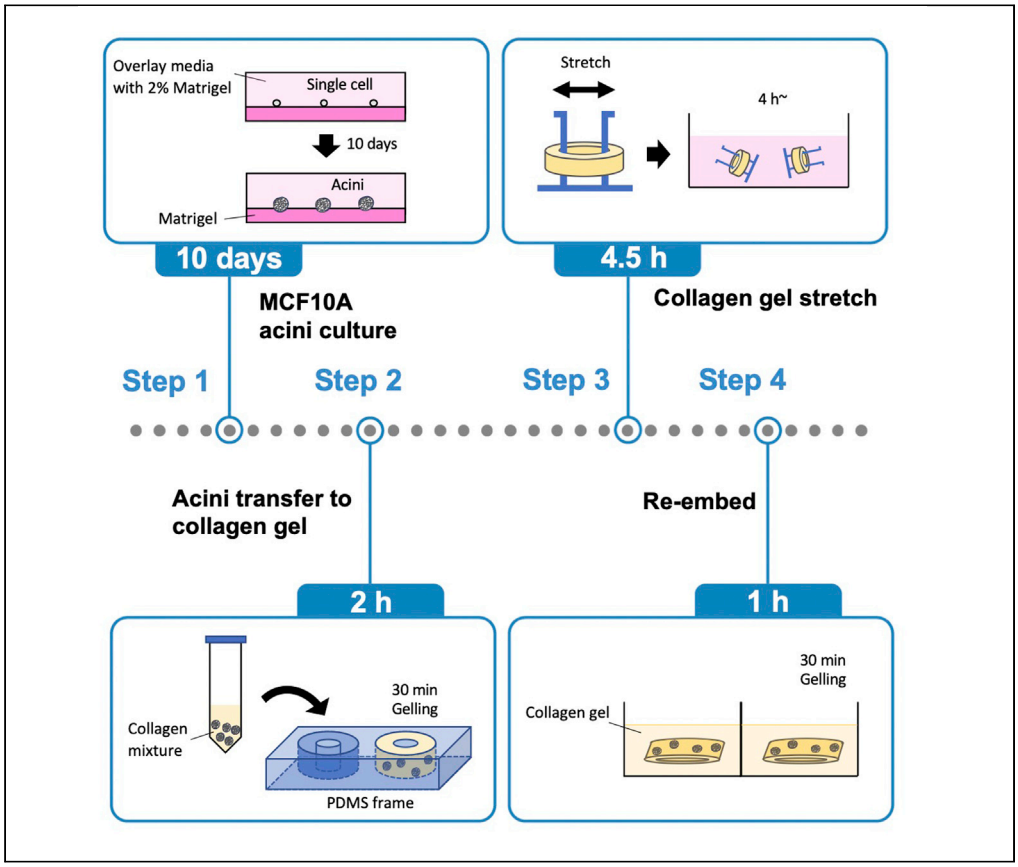
Protocol to transfer epithelial acini between different extracellular matrices and orient fibril organization



Extracellular matrix (ECM) provides fundamental support for epithelial tissues and controls cell function. The chemistry and mechanical properties of ECM components, including stiffness, elasticity, and fibrillar organization, influence epithelial tissue responses. Here we present a protocol describing the culture and transfer of epithelial acini from Matrigel to collagen gel and an approach to axially align the collagen fibrils by the external gel stretching. This protocol uses the acini of MCF10A cells and needs to be modified for different cell lines.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Hiroko Katsuno-Kambe, James E. Hudson, Holly K. Voges, Alpha S. Yap

hiro.katsukatsu@gmail.com (H.K.-K.)
a.yap@uq.edu.au (A.S.Y.)

Highlights

A protocol for transferring epithelial acini from Matrigel to collagen gel

Axial alignment of collagen fibrils by external gel stretching

Uses the acini of MCF10A cells and needs to be modified for different cell lines

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Protocol to transfer epithelial acini between different extracellular matrices and orient fibril organization

Hiroko Katsuno-Kambe,[1,3,*] James E. Hudson,[2] Holly K. Voges,[2] and Alpha S. Yap[1,4,*]

[1]Division of Cell and Developmental Biology, Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD 4072, Australia

[2]QIMR Berghofer Medical Research Institute, Brisbane, QLD 4006, Australia

[3]Technical contact

[4]Lead contact

*Correspondence: hiro.katsukatsu@gmail.com (H.K.-K.), a.yap@uq.edu.au (A.S.Y.)
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SUMMARY

Extracellular matrix (ECM) provides fundamental support for epithelial tissues and controls cell function. The chemistry and mechanical properties of ECM components, including stiffness, elasticity, and fibrillar organization, influence epithelial tissue responses. Here we present a protocol describing the culture and transfer of epithelial acini from Matrigel to collagen gel and an approach to axially align the collagen fibrils by the external gel stretching. This protocol uses the acini of MCF10A cells and needs to be modified for different cell lines. For complete details on the use and execution of this protocol, please refer to Katsuno-Kambe et al. (2021).[1]

BEFORE YOU BEGIN

Preparation of chemical reagents

Timing: ~0.5 h

1. Prepare 0.5 M NaOH and 0.2 M HEPES solutions to neutralize collagen mixture. Store them at 4°C.
2. Store PBS at 4°C. This will be used for washing out the Matrigel to transfer acini into the collagen gel.

Making PDMS casting well and stretching poles

Timing: 1 day

3. Preheat oven to 65°C and clean Teflon ring template and stretching pole polycarbonate template with 80% ethanol, followed by water and dry with compressed air.

   Note: Template designs and dimensions can be found in Voges et al.[2] and fabricated at local workshop facilities. The polydimethylsiloxane (PDMS) casting wells and stretching poles are also previously described in Voges et al.[2]

4. Mix PDMS elastomer at a 10 to 1 ratio (w/w) of base agent to curing agent (from the Sylgard 184 silicone elastomer kit).
   a. E.g., Slowly weigh out 30 g of PDMS base agent into large weigh boat.

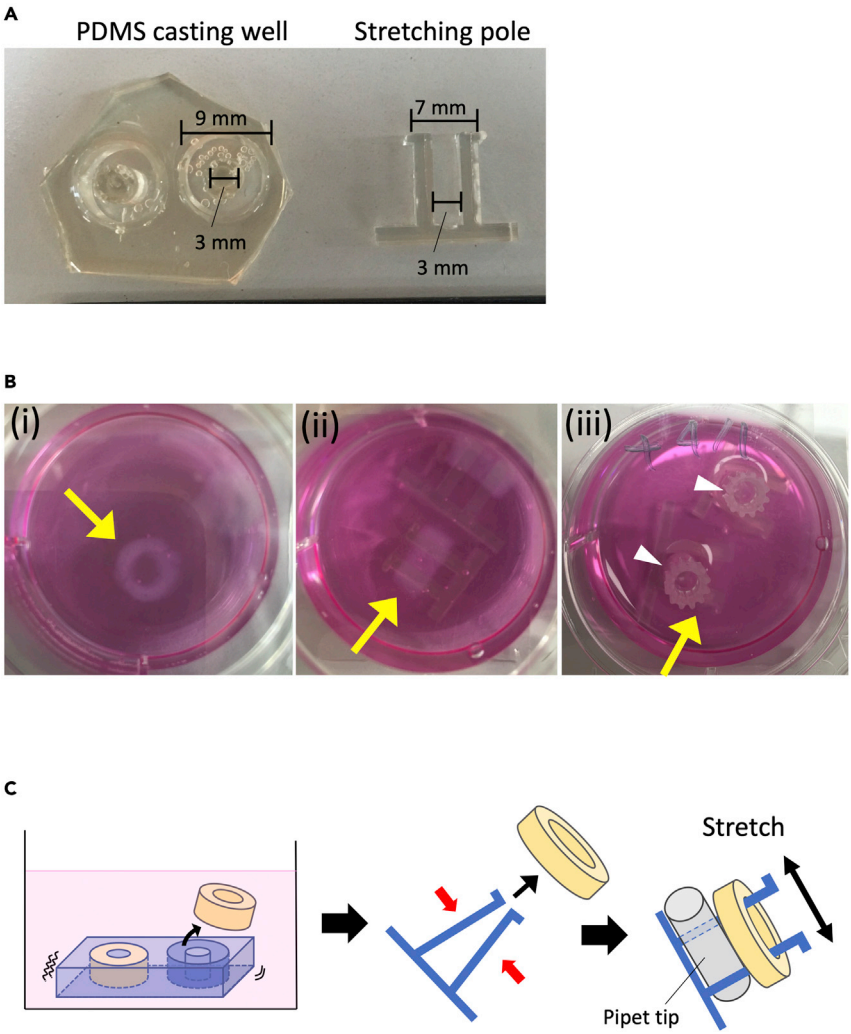

Figure 1. Stretching of the ring-shape collagen gel

(A) PDMS casting well and stretching pole for collagen gel stretching.

(B) The image of ring shape collagen gel which is stretched by stretching pole (yellow arrows). (i) Collagen gel which is removed from casting well. (ii) Collagen gel with inserted stretching pole. (iii) Stretching pole is expanded by the 10 μL pipet tip (white arrowheads).

(C) Cartoon illustrating how to insert the stretching pole to the collagen ring gel. Collagen gel is removed from PDMS casting wells by shaking in the medium. Stretching pole is pinched by tweezer (red arrows), and then inserted into the hole of the ring gel. 10 μL pipet tip is inserted between stretching pole to expand the pole and stretch the gel more.

   b. Weigh out 3 g of PDMS curing agent.
   c. Mix thoroughly with spatula for several minutes.
5. To remove bubbles from the PDMS elastomer, place weigh boat in the vacuum desiccator and vacuum for 30 min with regular repressurizing.
6. Once bubbles are removed, add 30 g of PDMS elastomer to the pole polycarbonate template and into wells of a 12 well plate to a height of approximately 0.5 cm. Wipe away any excess to ensure PDMS is level with the template edge.
7. Place 2 Teflon ring templates per well of a 12 well plate, ensuring a portion of the Teflon ring remains above the surface of the PDMS.
8. Return both the template and the 12 well plate with PDMS to the vacuum desiccator on a flat surface for 30 min.

Note: Visually inspect that no bubbles remain and use syringe to expel air directly above any remaining bubbles.

9. Place both template and 12 well plate onto a hotplate for 1 h to cure the PDMS. Ensure templates are level with spirit level.
10. Once cured the PDMS molds can be removed from templates.
    a. Remove the casting well from the 12 well plate using forceps and a scalpel by cutting around the outside of the well.
    b. Remove the stretching poles from the template by carefully peeling the PDMS away and cut the excess PDMS to leave clean individual pairs of poles.
11. Store stretching poles and casting wells at room temperature. Day before use, wash with 80% ethanol or autoclave to ensure sterility (Figure 1A).

Culture MCF10A acini

Timing: 10 days

12. Culture MCF10A cells on growth factor reduced Matrigel for 10 days until cells have grown to form lumenized acini as previously described.[3,4]
    a. Pre-coat 8 well chamber coverglass with 50 μL of 100% growth factor reduced Matrigel and incubate at 37°C in the CO_2 incubator for 30 min.
    b. Resuspend single isolated cells in assay medium containing 2% growth factor reduced Matrigel and 5 ng/mL of EGF (10,000 cells/well).
    c. Add 500 μL of solution in step 5b to pre-coated 8 well chamber coverglass.
    d. Incubate at 37°C in the CO2 incubator.
    e. 500 μL of Culture medium (assay medium) including 2% growth factor reduced Matrigel, 5 ng/mL of EGF are changed every 3–4 days.

⚠ CRITICAL: Use a pipet to change the medium. Do not to use an aspirator to remove old medium, because solidified Matrigel would be broken.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Ethanol undenatured	ChemSupply	EA043-2.5L-J
NaOH	MERCK	1064980500
HEPES	Astral Scientific	BIOHB0264
PBS	Astral Scientific	09-8912-100
DMEM/F12	Invitrogen	11330-032
Horse serum	Thermo	16050130
Cholera toxin	Sigma	C8052
Hydrocortisone	Sigma	H-0888
Insulin	Sigma	91077C
EGF	PeproTech	AF-100-15
Penicillin/Streptomycin	Invitrogen	15070-063
Growth factor reduced Matrigel	Corning	FAL354230
Collagen type 1	Corning	FAL354236
CNA35	Yap lab	N/A
Cell recovery solution	Corning	FAL354253

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Cell lines		
MCF10A cells (Passage number 10–35)	ATCC	CRL-10317 RRID: CVCL_0598
Other		
8 well chamber coverglass	Eppendorf	0030742036
6 well plastic plate	Corning	CLS3516-50EA
12 well plastic plate	Nunc	150628
15 mL tubes	Corning	CLS430791
2 mL tubes	Corning	0030120094
37°C CO_2 incubator	SNAYO	MCO-17-AIC
Forceps	Mabis	25-724-000
Scalpel	FEATHER	No.10
Teflon ring	Voges et al.[2] (manufactured by University workshop)	N/A
Vacuum desiccator	ProSciTech	PEL2240-1
Stretching pole polycarbonate template	Voges et al.[2] (manufactured by University workshop)	N/A
Microscope	Nikon	TS100
Sylgard 184 silicone elastomer kit, polydimethylsiloxane	Dow Corning	761036

MATERIALS AND EQUIPMENT

Growth medium	Final concentration	Amount
DMEM/F12	N/A	500 mL
Horse serum	5% v/v	25 mL
EGF (100 μg/mL)	20 ng/mL	100 μL
Hydrocortisone (1 mg/mL)	0.5 mg/mL	250 μL
Cholera toxin (1 mg/mL)	100 ng/mL	50 μL
Insulin (10 mg/mL)	10 μg/mL	500 μL
Pen/Strep (100×)	1×	5 mL
Total	**N/A**	**500 mL**
Store at 4°C, 2 months.		

Assay medium	Final concentration	Amount
DMEM/F12	N/A	500 mL
Horse serum	2% v/v	10 mL
Hydrocortisone (1 mg/mL)	0.5 mg/mL	250 μL
Cholera toxin (1 mg/mL)	100 ng/mL	50 μL
Insulin (10 mg/mL)	10 μg/mL	500 μL
Pen/Strep (100×)	1×	5 mL
Total	**N/A**	**515 mL**
Store at 4°C, 2 months.		

Assay medium + EGF + Matrigel	Final concentration	Amount
Assay medium	N/A	47.5 mL
Growth factor reduced Matrigel	2%	1 mL
EGF (100 μg/mL)	5 ng/mL	2.5 μL
Total	**N/A**	**50 mL**
Store at 4°C, 2 months.		

Note: Keep this medium without EGF and add EGF just before use.

Collagen mixture	Final concentration	Amount
Collagen type 1 solution (3–4 mg/mL)	1.5 mg/mL	400–500 μL
0.5 M NaOH	N/A	15–20 μL
0.2 M HEPES (pH8.5)	20 mM	100 μL
Growth medium	N/A	Up to 1 mL
Total	**N/A**	**1 mL**

Keep on ice.

Note: Collagen mixture cannot be kept for long time. Mix each solution before use.

Note: The concentration of commercial collagen solution is batch dependent. The amount of collagen must be calculated in function of the original concentration.

STEP-BY-STEP METHOD DETAILS

Transfer MCF10A acini from Matrigel to collagen gel

Timing: 2 h

MCF10A acini grown on Matrigel for 10 days are transferred into collagen gel.

1. Gently aspirate the medium from the well and wash with PBS once.

   Note: Do not use an aspirator to remove the medium from the well. Use a pipette and gently wash. Otherwise, acini can be sucked up by the aspirator.

2. Put 8 well chamber coverglass with cells on ice and add 500 μL of cold cell recovery solution to each well.
3. Incubate on ice for 30 min.

   Note: In this step, Matrigel may detach from the coverglass, but it is not a problem.

   Note: Do not incubate too long on ice. This would cause loss of the basement membrane surrounding the acini.

4. Aliquot 2–3 mL of cold PBS in 15 mL tube.
5. Gently mix the cell recovery solution and Matrigel 3–5 times by pipetting in the chamber well until Matrigel is dissociated.
6. Collect the mixed solution into the aliquoted PBS in step 4.
7. Add 500 μL of cold PBS to the well. Wash the well with PBS and collect the solution into the same tube in step 6. This step will increase the total number of the acini collected.

   Optional: To collect as many acini as possible, repeat step 7.

8. Spin down at 1,200 rpm for 5 min at 4°C. Remove the supernatant and re-suspend with 8–10 mL of cold PBS.

   Note: Leave a small amount of supernatant behind, rather than trying to remove all the supernatant. This ensures that the acini pellet is not aspirated.

9. Spin down at 1,200 rpm for 5 min at 4°C.
10. Remove the supernatant and resuspend with 500 μL of growth medium. Keep on ice.

Note: Leave a small amount of supernatant when remove it by aspirator, and then remove all supernatant by a pipet.

11. Add growth medium, 0.2 M HEPES and 0.5 M NaOH to the resuspension and mix well. Then add collagen type 1 solution and mix gently.

Note: The concentration and pH of commercial collagen type 1 solution is batch dependent, so it must be adjusted to the target values. Before starting the experiment, test how much amount growth medium and 0.5 M NaOH should be added to the collagen mixture in the small batch. pH of collagen mixture is checked by litmus paper and adjusted to around pH7.5.

Note: To resuspend acini with collagen mixture, add collagen type 1 solution in the last step. This makes it easy to mix the viscous solution.

⚠ CRITICAL: The mixture which includes collagen must always be kept on ice before gelling.

⚠ CRITICAL: Do not vortex or mix vigorously; this is to avoid bubbles appearing in the solution.

Optional: Add the fluorescent labeled CNA35 at 2 μM in step 11 to label collagen fibers for visualization.

12. Fill 200–250 μL of collagen/acini solution from step 11 into the PDMS casting wells. Allow the collagen to solidify in the incubator at 37°C for 30 min.

Note: Put the collagen solution with acini in the incubator immediately after filling the PDMS casting wells. Acini will sink to the bottom if the solution is kept at room temperature for too long before gelling.

13. Aliquot 3–5 mL of growth medium in a 6 well plate and keep in the incubator at 37°C to warm up.

Stretch the collagen gel

Timing: 4.5 h

Ring shape collagen gels are stretched to align collagen fibrils.

14. Put the PDMS casting wells with collagen gel in the medium aliquoted in step 13.
15. Gently shake the PDMS casting wells in the medium with a tweezer and remove the collagen ring gel from the casting wells (Figures 1B and 1C).
16. Carefully insert the PDMS stretching poles inside the gel hole by using a tweezer (Figures 1B and 1C).
17. Insert the 10 μL pipet tip which is cut the tip between the stretcher pole to expand the gel (Figure 1B).
18. Incubate at 37°C for 4 h in the CO_2 incubator.

Note: All steps are done in suspended condition in growth medium.

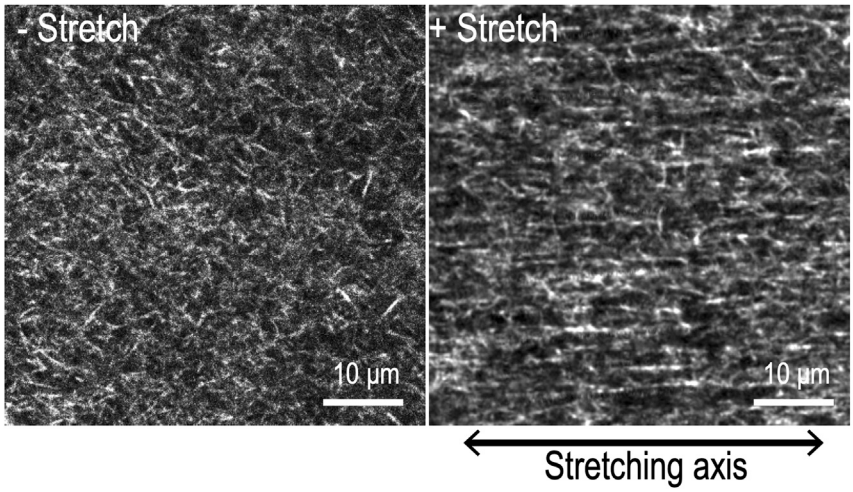

Figure 2. Collagen fibrils with or without gel stretching
Images are obtained by SHG microscopy and cited from Katsuno-Kambe et al.[1]

Re-embed the stretched collagen gel

Timing: 1 h

Stretched ring shape collagen gels are re-embedded in the collagen gel.

19. Make 1.5 mL/mL collagen mixture with growth medium and neutralize with 0.5 M NaOH and 0.2 M HEPES on ice.
20. Remove the inserted pipet tips and stretcher from the ring gel.
21. transfer the stretched gel in a 8 well chamber slide. Remove extra medium as much as possible.
22. Add 200–250 μL of collagen mixture made in step 19.

⚠ CRITICAL: Make sure that the stretched gels are completely covered by the newly added collagen solution.

⚠ CRITICAL: Do not leave and float the stretched gel after removing the stretching poles. The aligned collagen fibrils would be reversed.

23. Incubate at 37°C for 30 min in the CO_2 incubator.
24. Add 500 μL of growth medium for each well.

Note: Aligned collagen fibrils can be observed any time after step 24.

EXPECTED OUTCOMES

If collagen gels are stretched, individual collagen fibrils in the gel should be aligned along the stretched direction (Figure 2). Collagen fibrils can be visualized by confocal microscopy when collagen is labeled with fluorescent-tagged CNA35 or by second harmonic (SHG) microscopy without any staining.

In MCF10A cells, acini should start to elongate along the stretched axis of the gel within 24–48 h.

LIMITATIONS

We used the acini of MCF10A cells for this method. MCF10A cells adhere to each other and are surrounded by a thin layer of basement membrane. For this reason, acini are not individual single cells

during the process of transplantation. It is possible that acini formed with other cell lines break their structures during transplantation, so the current protocol needs to be customized for each cell line.

MCF10A cells can form acini-like structures both on Matrigel and in collagen gel, however, cells have proper polarity only when they are cultured on Matrigel. For this reason, we first cultured MCF10A cells on Matrigel and then transfer them into collagen gel. If cells can grow as acini or aggregate structures properly in collagen gel, cells can be directly cultured in ring-shape collagen gel and proceeded for stretching without the gel transfer step.

TROUBLESHOOTING

Problem 1

Few or no acini are transferred to collagen gel.

Potential solution

Wash the chamber coverglass well with PBS after incubating with cell recovery solution and collect all washed PBS solution in 15 mL tube (step 7). Check the chamber by microscope to assess whether acini have detached from the coverglass.

This also might happen if acini are vacuumed with the supernatant after spin down. To avoid this, leave 100–200 μL of solution when aspirating the supernatant, and then use the pipetman to gently remove the supernatant.

Problem 2

Collagen ring gels are broken.

Potential solution

Soft gels tend to break easily during the process (steps 15–17). In our hands, the lowest concentration of collagen used for this protocol is 1.5 mg/mL. The higher concentration of collagen gels should be much stiffer and easier to handle.

Problem 3

Collagen fibrils are not aligned after stretching.

Potential solution

Insert the small bar between the stretcher to expand the stretcher poles or use wider stretcher (step 17). We used 10 μL pipet tips to expand the stretcher poles (Figure 1B).

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to the Lead Contact, Alpha S. Yap (a.yap@uq.edu.au).

Materials availability

No unique reagents were generated.

Data and code availability

No large dataset or new software were developed.

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AUTHOR CONTRIBUTIONS

Investigation, H.K.; writing, H.K., H.V.; supervision, A.S.Y.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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