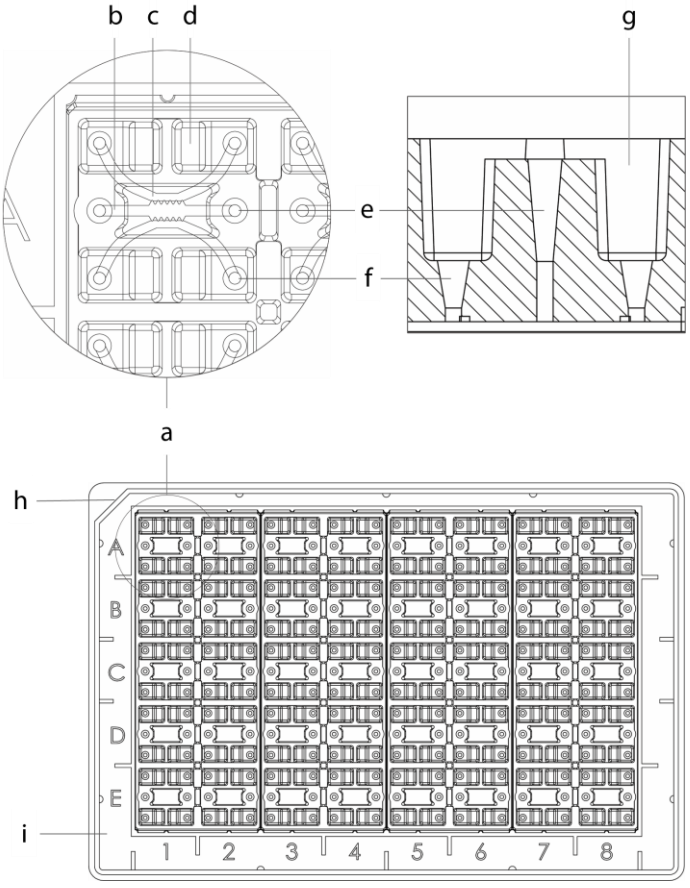
INTRODUCTION

The idenTx 40 is a sterile, single-use, and stackable plate, with a throughput size of 40. The perimeter of the plate serves as a reservoir to provide a humidified environment. This protocol covers the basic handling of the idenTx 40 – the preparation and filling of collagen gel, hydrating and coating of media channels, seeding cells, changing medium and staining cells. The formation of an endothelial monolayer is used here as illustration.

SCHEMATIC

The following schematic shows the 3D presentation of idenTx 40. This nomenclature will be used extensively in this protocol.



Nomenclature:

a : site (40 sites per plate)
b : gel channel
c : media channel
d : trough
e : gel inlet
f : media inlet
g : media port

h : chamfer
i : reservoir

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PREPARING & FILLING COLLAGEN GEL ⏱ TIMING 60 min

MATERIALS

Reagents

- 10 X PBS with phenol red (see REAGENT SETUP; Life Technologies, Cat. No. 70011044)
- Sodium hydroxide solution, 0.5 M (see REAGENT SETUP; Sigma-Aldrich, Cat. No. 221465)
- Sterile deionized water (Thermo Water Purifying System)
- Collagen type I, rat tail (Corning Life Science, Cat. No. 354236)
- Cell culture medium (Lonza, Cat. No. CC3202)

Others

- 1.5 ml microcentrifuge tube
- Pocket size pH meter (Hach, Cat. No. H138) or pH papers (Sigma Aldrich, Cat. No. 37144)
- Ice bucket or styrofoam box
- Ice
- idenTx 40
- AIM Cooling Block
  - *provides better contrast for each individual site and its channels*

Calculations before Experiments

i. The following steps are calculated based on a collagen stock solution of 3.75 mg/ml, but any other concentration in the range of 3 - 4.5 mg/ml may still be used to make 400 µl of 2.5 mg/ml collagen solution at pH 7.4. This amount is sufficient to fill 1 full idenTx 40.

Reminder *The concentration, pH value and total volume of the final collagen solution can be adjusted for different applications. Please see Table 1 for a list of publications that use different formulations of collagen gel for different experiments.*

ii. The volume of 10 X PBS with phenol red is always one tenth (1/10) of the final volume (400 µl), A = 40 µl

iii. The volume of collagen, B is calculated based on the equation below, B = 266.7 µl

$$3.75\ \frac{mg}{ml} \times B = \frac{2.5\ mg}{ml} \times 400\ \mu l$$

iv. The volume of 0.5 M NaOH needed to adjust the pH of the solution is a variable that usually falls between 8 to 16 µl and the exact number can be determined through an iterative method (see Table 1). For a collagen stock solution of 3.75 mg/ml, the volume of 0.5 M NaOH solution needed, C = 15.6 µl

v. Add sterile deionized water to dilute the collagen solution to the desired concentration without affecting the pH value. The volume of deionized water, D is calculated last by using the equation below.

$$D = Total\ Volume - A - B - C$$

$$D = 400\ \mu l - 40 \mu l - 266.7\ \mu l - 15.6\ \mu l = 77.7\ \mu l$$

Table 1 List of applications with different formulations of collagen gel (or other hydrogel)

Main Category	Sub-category	Collagen I Gel Concentration	References
Vascular functions	Angiogenesis	2.5 mg/ml at pH 7.4	[1]
		2.5 mg/ml at pH 7.4	[2]
		2.5 mg/ml at pH 7.4	[3]
		2.0 mg/ml at pH 7.4	[4]
	Anti-angiogenesis	3.0 mg/ml at pH 7.4	[5]
	Vasculogenesis	2.0 mg/ml, 2.5 mg/ml and 3.0 mg/ml at pH 7.4; Fibrinogen only and mixture of both	[6]
	Flow response	2.5 mg/ml at pH 7.4	[7]
	Transendothelial migration	2.0 mg/ml at pH 5, pH 7.4 and pH 11	[8]
Cancer	Spheroid dispersion	2.5 mg/ml at pH 7.4	[9]
	Extravasation	2.0 mg/ml	[10]
		Fibrinogen only: 2.5 mg/ml and 5 mg/ml	[11]
		6.0 mg/ml	[12]
	Intravasation	2.5 mg/ml	[13]
	Flow response	2.0 mg/ml at pH 8.9	[14]
		2.0 mg/ml at pH 8.9	[15]
	Invasion and Migration	2.0 mg/ml at pH 7.4	[16]
		2.0 mg/ml at pH 11, Matrigel only and mixture of both	[17]
		2.0 mg/ml at pH 6.0, pH 7.4 and pH 11	[18]
Neurobiology	Neurite guidance	2.0 mg/ml at pH 7.4	[19]
	Differentiation of NSC	2.0 mg/ml at pH 7.4 + Matrigel (1:1)	[20]
Stem cell Biology	Differentiation of ESC	2.0 mg/ml at pH 7.4	[21]

Iterative method to determine the volume of 0.5 M NaOH solution

1. Perform the following steps in a laminar flow hood and every item should be sterilized beforehand.

2. Keep 10X PBS with phenol red, 0.5 M NaOH solution, deionized water and collagen stock solution on ice throughout the process.

Reminder *Make sure the color of 10X PBS with phenol red is homogeneous before use as the color of 10X PBS with phenol red changes when it is about to freeze.*

3. Add 40 µl of 10 X PBS with phenol red into a microcentrifuge tube (on ice).

4. Add the calculated volume of collagen, B into the microcentrifuge tube.

5. Add an estimated amount of 0.5 M NaOH solution into the microcentrifuge tube. The recommended starting volume is 8 µl to make 400 µl of 2.5 mg/ml collagen solution at pH 7.4.

6. Mix thoroughly with a micropipette.

! Critical *Pipette up and down until the color is homogeneous throughout (if unsure, pipette at least 100 times).*

! Critical *Pipette up and down with care to avoid the generation of bubbles.*

! Critical *Keep the microcentrifuge tube on ice while mixing to prevent any unwanted polymerization of the collagen gel.*

7. Check the color of the mixed solution. Phenol red is a pH indicator itself and the desired color for pH7.4 is faint pink.

8. Discard the mixed solution if the mixed solution is purple or red, which indicates a high pH value. Start again with a lower volume of 0.5 M NaOH solution.

9. Add 0.5 M NaOH solution into the mixed solution in a small step wise manner (each addition is not more than 0.5 µl) if the mixed solution is yellow, which indicates a low pH value. Mix thoroughly with each addition of 0.5 M NaOH solution. Repeat this until the color changes to faint pink.

! Critical *Make sure no residue is left in the pipette tip as the pH value is highly sensitive to the addition of 0.5 M NaOH solution.*

10. If a pocket size pH meter is available, add a drop of mixed solution (~10 µl depends on the model of pH meter) to measure the pH value. Otherwise, add 10 µl to 20 µl of mixed solution on a pH paper and match it to the color chart.

11. Confirm the pH value is within ±0.1 of the target value with a pH meter. Otherwise, make sure the color of mixed solution is faint pink and the pH paper readout lies between pH7 and pH8.

12. Calculate the volume of deionized water required and add it into the mixed solution.

13. The collagen gel recipe may be used for future experiments as long as the same reagents are used. For any change of reagent, a new recipe has to be developed again.

Table 2 An example of collagen gel recipe

Reagents	Volume
10X PBS	40 µl
Collagen	266.7 µl
0.5 M NaOH	15.6 µl
Deionized water	77.7 µl

Preparing collagen gel ⏱ **TIMING 10 min**

14. Keep 10X PBS with phenol red, collagen stock solution, 0.5 M NaOH solution and deionized water on ice and add them into a microcentrifuge tube (on ice) sequentially according to the pre-determined collagen gel recipe.

15. Mix the solution thoroughly by using a micropipette to get a collagen solution with homogeneous faint pink color.

Filling collagen gel ⏱ **TIMING 20 min + 30 min incubation**

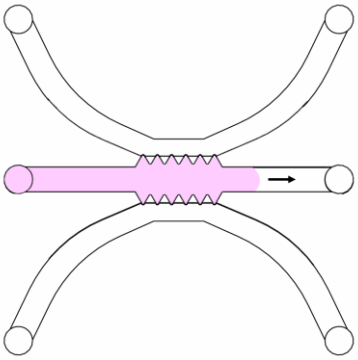
16. Remove the idenTx 40 from the packaging.

Reminder *The AIM Cooling Block may be used to provide better contrast and keep unpolymerized collagen chilled before incubation. The Cooling Block is best used when placed on ice.*

17. Draw 5 µL of collagen solution with a 1- 10 µL micropipette. Make sure the collagen solution is kept on ice at all times.

! Critical *Limit the volume of collagen solution to 5 µl to prevent the collagen solution from overflowing into the media channels.*

18. Insert the micropipette tip into either gel inlet. Push the plunger slowly to inject the collagen gel until it reaches the other inlet.



! Critical *Hold the plunger firmly while removing the micropipette from the inlets, otherwise the negative pressure will suck the collagen up.*

! Critical *Avoid discharging collagen solution abruptly to prevent the collagen solution from overflowing into media channels.*

? Troubleshooting (see Table 3 for troubleshooting advice)

19. Distribute up to 6 ml of sterilized water across the perimeter of the reservoir to ensure full coverage.

! Critical *Be careful not to tilt the plate when the reservoir is filled to avoid spilling of water into the sites.*

20. Incubate the idenTx 40 in a 37 °C incubator for 30 min to allow the polymerization of collagen.

! Critical *Be careful while handling to avoid spilling of gel into the media channels.*

HYDRATING & COATING MEDIA CHANNELS ⏱ TIMING 20 min + 60 min incubation

MATERIALS

Reagents

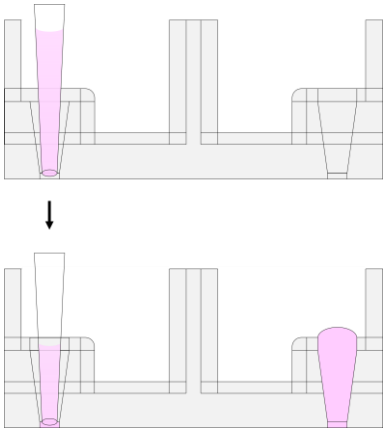
- Fibronectin (Sigma-Aldrich, Cat. No. F0895) or any coating reagent for your specific application

! Critical *Collagen coating is not suitable for collagen-filled idenTx 40 as the solvent of the collagen coating solution may dissolve the polymerized collagen.*

Others

- Collagen-filled *idenTx 40*

21. After incubation, insert a pipette tip into either inlet of the media channel that requires coating and push gently until the tip fits. Inject 10 µl of coating solution (e.g. 50 µg/ml fibronectin solution diluted in culture medium or 1X PBS) into the channel. Due to surface tension, the injected coating solution will form a spherical cap at the opposite inlet. Repeat this step for the other channel. Use culture medium to hydrate the media channels if coating is not required.



Insert a tip into an inlet until it fits. Inject medium till it reaches the opposite inlet.

! Critical *Hold the plunger firmly while removing the micropipette from the inlets, otherwise the negative pressure will suck the coating solution/medium up.*

! Critical *Do not inject more than 10 µl of coating solution/medium at this step or the high injection pressure may disrupt the collagen gel.*

? Troubleshooting (see Table 3 for troubleshooting advice)

22. Incubate the media-channel-hydrated sites for 1 h in a 37°C incubator.

23. Add 70 µl of medium into one port and then add 50 µl into the opposite port of the same media channel to flush out the coating solution. Repeat this for the other channels.

    PAUSE POINT *The media-channel-hydrated plate can be kept in an incubator for not more than 2 days before the seeding steps depending on your application (In a single experiment, do not mix the media-channel-hydrated plate that are prepared at different time points because the properties of the collagen may change over time).*

Reminder *Media channels must be hydrated with culture medium/coating solution after the polymerization of collagen. This is to prevent the collagen gel in the plate from drying up.*

Reminder *If the coating solution has to be removed completely, remove all media from the ports and add 120 µl of medium into 1 port. Repeat this for another channel.*

SEEDING CELLS ⏱ TIMING 30 min + 60 min incubation

MATERIALS

Reagents

- 1X PBS (Life Technologies, Cat. No. 70011044)
- 0.25% trypsin with EDTA (Lonza, Cat. No. CC5012)
- Cell culture medium (Lonza, Cat. No. CC3202)

Others

- Collagen-filled and fibronectin-coated IdenTx 40
- Cells
- Centrifuge

24. Trypsinize cells as per protocol. Briefly, remove medium from the cell culture flask/dish and wash the endothelial cells with sterile 1X PBS for twice. Add enough 0.25 % trypsin with EDTA solution to cover the bottom of the culture flask/dish and incubate them for 2 min in a CO_2 incubator.

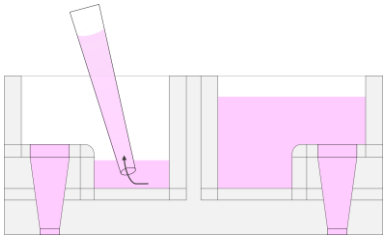
25. Perform a visual inspection to make sure the cells have detached from the substrate.

26. Add medium with FBS, at least 5 times the volume of trypsin, into the culture flask/dish to neutralize the activity of trypsin.

27. Transfer the cell suspension to a 15 ml tube and pellet the cells by centrifuging at 250 x*g* for 5 min at RT.

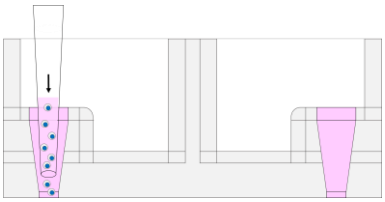
28. Re-suspend the cells in culture medium with densities ranging from 1 M to 3 M cells/ml, depending on cell types and applications. In this example using endothelial cells, 5 µl of 2 M cells/ml is sufficient to form a confluent monolayer overnight.

29. Remove medium from all 4 ports by carefully aspirating the medium from the troughs. Doing so prevents backflow or overflow of cells into the media ports during cell seeding (Step 30).



! Critical *Do NOT aspirate medium from inlets to avoid accidental removal of medium from the channels.*

30. Use a micropipette to withdraw 5 µl of the endothelial cell suspension (2 M cells/ml). Position the tip in the media channel inlet and inject the cell suspension slowly (e.g. over a duration of 3 s).



! Critical *Do not insert the tip completely into the inlets to avoid introducing cells into the media channels at a high flow rate. High flows will not allow cells to settle along the channel, resulting in uneven distribution.*

! Critical *Lay the idenTx 40 on a flat surface while seeding cells into the idenTx 40. Inclination of the plate affects the flow in the media channel, thus disturbing cell distribution.*

31. Visual inspection of the channel under a microscope is recommended. If the cell density or distribution is not optimal, add 120 µl of culture medium to 1 media port to flush out the cells. Adjust the cell concentration and repeat Steps 29 – 30.

32. Keep the idenTx 40 in a 5 % CO_2 incubator at 37°C for at least 1 h to allow the endothelial cells to adhere.

33. If cells are to be seeded on the other media channel, repeat Step 30. If not, proceed to Step 34.

34. Add 120 µl of culture medium into 1 media port. The wetted media ports will allow the culture medium to flow through the channel and equilibrate. This can be observed by the medium build-up in the opposite port. (TROUBLESHOOT)

? Troubleshoot *See Table 3 for the alternative method if media did not flow through to the opposite port.*

35. Keep the idenTx 40 in a 5 % CO_2 incubator at 37°C. Proceed to Step 36 for the changing of media.

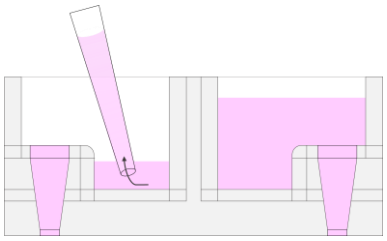
CHANGING MEDIUM ⏱ TIMING 15 min

MATERIALS

Reagents

- Cell culture medium (Lonza, Cat. No. CC3202)

36. Remove medium from all 4 ports by carefully aspirating the medium from the troughs.



! Critical *Do NOT aspirate medium from inlets to avoid accidental removal of medium from the channels.*

37. Replace the medium by adding 120 µl of culture medium into 1 media port. Ensure that the medium builds up on the opposite media port to indicate a successful medium change.

? Troubleshoot *See Table 3 for the alternative method if media did not flow through to the opposite port.*

38. Repeat for the opposite media channel.

39. Keep the idenTx 40 in a 5 % CO_2 incubator at 37°C. Endothelial cells should form a confluent monolayer covering the channel in 1 d. If the cells need to be kept longer in culture, change the medium daily.

STAINING CELLS ⏱ TIMING 2 d

MATERIALS

Reagents

- 4% Formaldehyde (See REAGENT SET UP; Sigma Aldrich, Cat. No.158127)
- 0.1% Triton X-100 (See REAGENT SET UP; Sigma Aldrich, Cat. No. T8787)
- Blocking buffer (Life Technologies, Cat. No. B-10710)
- 1X PBS (Life Technologies, Cat. No. 70011044)
- Primary Antibody: VE-cadherin (Enzo Life Sciences, Cat. No. ALX-210-232-C100)
- Corresponding Secondary Antibody (Life Technologies, Cat. No. A11034)
- Rhodamine Phalloidin (Life Technologies, cat. No. R415)
- Hoechst (Life Technologies, Cat. No. H1399)

Others

- Parafilm

Cell Fixation ⏱ TIMING **20 min + 15 min incubation**

40. Remove the medium from all 4 ports carefully by placing the tip of an aspirator at the troughs.

41. Add 120 µl of 1X PBS into one port and observe for fluid build-up in the opposite connected port to indicate a successful reagent exchange. Repeat this for the other channel.

42. Remove 1X PBS from all ports. Add 120 µl of 4% formaldehyde into one of the ports. Repeat this for the other channel.

43. Incubate for 15 min at RT.

44. Remove 4% formaldehyde from all ports by using a micropipette and wash the channels twice with 1X PBS as described in step 39.

! Critical *Dispose 4% formaldehyde and the 1st subsequent wash in a designated waste bottle.*

▪ **PAUSE POINT** *Fixed cells can be kept for up to 1 month (depending on target antigens as some antigens may degrade over time after fixation) at 4°C as long as the PBS in the ports does not dry up.*

Cell Permeabilization ⏱ TIMING **15 min + 10 min incubation**

(Optional: Permeabilization is only necessary when cell-impermeable fluorescent probes are used, e.g. phalloidin)

45. Remove PBS from all 4 ports carefully by placing the tip of an aspirator at the troughs.

46. Add 120 µl of 0.1 % Triton X-100 into one of the ports. Repeat for the other channel.

47. Incubate for 10 min at RT.

48. Wash once with PBS as described in step 43.

Blocking ⏱ **TIMING 15 min + 120 min incubation**

(Optional: Blocking is only necessary for immunofluorescent staining)

49. Remove PBS from all 4 ports carefully by placing the tip of an aspirator at the troughs.

50. Add 120 µl of blocking buffer into one of the ports. Repeat this for the other channel.

51. Incubate for 2 h at RT.

Primary Antibody Staining ⏱ **TIMING 18 h**

(Optional: Primary antibody staining is only necessary for immunofluorescent staining)

52. Prepare antibodies according to the manufacturer's recommendations. For example, dilute the anti-VE-cadherin antibody with 1X PBS in a 1:100 ratio.

53. Remove blocking buffer from all 4 ports carefully by placing the tip of an aspirator at the troughs.

54. Add 120 µl of antibodies into one of the ports. Repeat this for the other channel. If the target cells are embedded within the 3D hydrogel, apply differential volumes of antibodies between media channels (e.g., 120 µl in one channel and 80 µl in the other) to generate interstitial flow that can transport the antibodies to the cells.

! Critical *Do not wash with 1X PBS after the blocking step.*

55. Seal idenTx 40 with parafilm. Incubate overnight at 4°C.

56. Remove the primary antibodies from all 4 ports carefully by placing the tip of an aspirator (or using a micropipette to recycle the antibodies) at the troughs. You may reuse the antibodies depending on the antigen and antibody.

57. Wash the channels with 1X PBS 5 times, with a 5 min incubation between each wash, as described in step 43.

Secondary Antibody Staining ⏱ **TIMING 30 min + 60 min incubation**

(Optional: Secondary antibody staining is only necessary for immunofluorescent staining)

58. Prepare the secondary antibodies according to the manufacturer's recommendations. For example, dilute the Alexa Fluor-conjugated goat anti rabbit-secondary antibody with 1X PBS in a 1:100 ratio.

59. Add 120 µl of antibodies into one of the ports. Repeat this for the other channel. If the target cells are embedded within the 3D hydrogel, apply differential volumes of antibodies between media channels (e.g., 120 µl in one channel and 80 µl in the other) to generate interstitial flow that can transport the antibodies to the cells.

! Caution *Cover the idenTx 40 with aluminium foil to minimize photobleaching.*

60. Incubate for 1 h in the dark at RT.

61. Remove the secondary antibodies from all 4 ports carefully by placing the tip of an aspirator (or using a micropipette to recycle the antibodies) at the troughs. You may reuse the antibodies depending on the antigen and antibody.

62. Wash the channels with 1X PBS 3 times, with a 5 min incubation between each wash, as described in step 43.

Fluorescent Staining ⏱ **TIMING 1.5 h**

63. Remove PBS from the ports and then add 120 µl of Hoechst (10 µg/ml)/Rhodamine Phalloidin (3 U/ml) into one of the ports. Repeat this for the other channel.

Reminder *The fluorescent staining of nuclei and actin by using Hoescht and Rhodamine Phalloidin can be carried out either concurrently or separately.*

! Caution *Hoechst (or other nucleus staining reagents) should always be handled using protective gloves and clothing.*

64. Incubate for 1 h in the dark at RT.

Reminder *Incubation time for actin staining can be increased if stronger fluorescent intensity is needed.*

65. Wash the channels with 1X PBS 5 times, with a 5 min incubation between each wash as described in step 43. Seal the idenTx 40 with parafilm and keep them in 4 °C and protect them from light until imaging.

Reminder *The staining protocol should be optimized for your specific application (with different cell types and different proteins of interest).*

▪ **PAUSE POINT** *Stained cells can be kept for up to 1 month (depending on your application) at 4°C in dark as long as the PBS in the ports does not dry up.*

TROUBLESHOOTING

Table 3 Troubleshooting advice

Step	Problem	Possible Reason	Solution
18.	Collagen solution overflows into the media channels	Filling pressure is too high	Inject collagen solution smoothly
		Filling volume is more than required	Use 10 µl of collagen solution only
		Collagen polymerizes thus blocking flow	Handle collagen solution on ice and avoid prolonged injection
21.	Medium leaks from one channel to the other channel	Insufficient polymerization time	Increase the polymerization time
		Collagen quality may vary from manufacturer to manufacturer and from batch to batch	Change to a new collagen stock from a different batch or manufacturer
23.	Air bubbles are trapped in the media channels	Humidity level is not maintained high during hydrogel polymerization	Ensure the reservoir is filled and avoid prolonged polymerization time
30.	Cells do not distribute evenly	The injection speed is inconsistent, resulting in cells either flowing too quickly or too slowly into the media channel	Press the plunger slowly and smoothly over 3 s.
30.	Too many cells in a channel	Concentration of cell suspension is too high	Flush out unattached cells with culture medium immediately and repeat the seeding steps with cell suspension that is less concentrated
30.	Too few cells in a channel	Concentration of cell suspension is too low	Increase the concentration of cell suspension or repeat the seeding steps (without modifying the concentration of cell suspension) until the target cell density is obtained
30.	Cells do not adhere to the gel interface	The pressure head applied is insufficient	Increase the volume of cell suspension
37.	Medium did not flow through and build up in the connected opposite media port	The media port is too dry or not wetted	Remove medium. Add 70 µl of medium into 1 media port, then 50 µl into the connected opposite port

REAGENT SETUP

0.5 M SODIUM HYDROXIDE (NaOH) SOLUTION

Reagents

- Sodium Hydroxide (Sigma Aldrich, Cat. No. 221465)
- Deionized water (Thermo Fisher Water System)

Others

- 0.2 µm 250 ml bottle top filter or 0.2 µm syringe filter

1. Dissolve 2 g of NaOH (molecular weight: 40 g/mol) pellets in 90 ml of deionized water by stirring at room temperature. Top up the solution with deionized water to yield final volume of 100 ml (final concentration is 0.5 M). Sterilize NaOH solution by passing through a 0.2 µm bottle top filter or syringe filter in a sterile laminar flow hood.

2. Seal and keep the filtered NaOH solution at 4 °C for collagen gel preparation. Aliquot the solution into smaller volume (e.g. 1 to 5 ml) is recommended as the amount of NaOH solution needed for each experiment is small (typically ranging between 5 µl to 20 µl, depending on the total amount of collagen gel).

! Caution *Wear protective clothing and gloves while working with NaOH. Avoid the addition of large amount of NaOH into water at once to prevent the generation of excessive heat from this exothermic reaction.*

10 X PBS WITH PHENOL RED

Reagents

- 10X PBS (Life Technologies, Cat. No. 70011044)
- Phenol red sodium salt (Sigma-Aldrich, Cat. No. 114537)

Others

- 250 ml 0.2 µm bottle top filter or 0.2 µm syringe filter
- Vortex mixer

3. Dissolve 63.6 mg of phenol red sodium salt into 40 ml of 10X PBS as primary stock solution through vortexing. Properly sealed primary stock solution is stable at room temperature for up to a year.

4. Dilute primary stock solution with 10X PBS in a 1: 10 ratio (5 ml of primary stock solution + 45 ml of 10X PBS) to yield the working concentration. Sterilize the 10X PBS solution (with phenol red) by passing the solution through a 0.2 µm bottle top filter or a syringe filter in a sterile laminar flow hood.

5. Seal and keep the filtered 10X PBS with phenol red at 4 °C for collagen gel preparation. Aliquot the solution into smaller volume (e.g. 1 to 5 ml) is recommended as the amount of 10X PBS with phenol red needed for each experiment is small (typically ranging between 20 µl to 40 µl, depending on the total amount of collagen gel).

4% FORMALDEHYDE

Reagents

- Paraformaldehyde powder (Sigma-Aldrich, Cat. No. 158127)
- 1X PBS (Life Technologies, Cat. No. 70011044)

Others

- Hot plate with magnetic stirrer

6. Add 40 g of paraformaldehyde (PFA) powder into 800 ml of 1X PBS and heat it up to 60 °C by using a hot plate.

! Caution *Wear protective clothing and gloves while working with PFA. Prepare this solution in a ventilated hood.*

7. Stir for approximately 6 h.

Reminder *pH can be adjusted by using 1 M NaOH to facilitate the dissolution of PFA. If so, neutralize the pH back to approximately pH 7.0 by using dilute HCL after the PFA is dissolved.*

8. Adjust the volume to 1L with 1X PBS and then filter the solution and make aliquots.

0.1% TRITON-X

Reagents

- Triton X-100 (Sigma Aldrich, Cat. No. T8787)
- 1X PBS (Life Technologies, Cat. No. 70011044)

9. Dilute Triton X-100 with 1X PBS to yield 0.1 % (v/v) working concentration.

10. Aliquot the solution and store them at room temperature.

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