User Manual to Pattern Cells in Fibrin

Purpose

This protocol outlines the steps for successful cell patterning in **Fibrin** by use of mimiX labware and cymatiX®, equipped with the cooling platform. It provides guidance for standard cell patterning and highlights the most important DOs and DON'Ts. Please adjust according to your needs and experimental questions.

Additional Protocols needed:

General Protocols

- User Manual cymatiX®
- Protocol *Frame Preparation and Patterning Parameters*

1. Description of Fibrin Hydrogels

Fibrin is a natural hydrogel that is formed when fibrinogen is mixed with thrombin. The enzymatic gelation is initiated immediately and takes about 4 minutes to completion at room temperature. Gelation can be slowed down if working at a lower temperature of e.g. 16°C. Fibrinogen and thrombin are natural products, derived from human plasma, and as such present batch to batch variability. For reconstitution after purchase, it is important to check the certificate of analysis for the fraction of clottable protein of fibrinogen and the activity in International Units of thrombin.

Table 1.1 *Chemicals needed for Fibrin hydrogels*

Item #	Name	Catalogue #	Storage	Distributor	Hazards
1	Fibrinogen, reconstituted at 30 $mg \cdot mL^{-1}$, at 82% clottable protein fraction	f4883-500 mg	-20°C	Sigma	
2	Thrombin, reconstituted at 100 $IU \cdot mL^{-1}$	T4393-100 UN	-20°C	Sigma	

1.2 Additional chemicals and materials needed

- Expanded cells, depending on the experiment
- General cell lab consumables, including PBS, filters and syringes.
- reaction vials, e.g. Eppendorf tubes.
- NaCl, $CaCl_2$
- mimiX frames
- TPP petri dishes, 60 mm in diameter, catalogue #93060

2. Preliminary checks and preparation

- *Initiate the* cymatiX® instrument *and run the automatic test*
- *Calibrate all necessary items in accordance with the user manual*
- *If needed, sterilise labware and instruments beforehand.*

Reconstitute thrombin in sterile filtered 1.1 % (w/v) NaCl in 2 mM CaCl2
Prepare aliquots at 100 IU/mL and store at -20°C
1 NIHunit = 1.15 IU
With the lot number, the NIHunit for each vial can be checked on the Sigma homepage and the powder reconstituted accordingly..

Reconstitute Fibrinogen in sterile filtered 1.1 % NaCl
Prepare stock solutions of 30 mg·mL^{-1} at a clottable protein fraction of 82%.
The clotting ratio depends on the lot and can be checked on the Sigma homepage (can be as low as 50%); it is indicated in the product analysis sheet. Reconstitute Fibrinogen accordingly. Aliquot and store at -20°C.

3. Procedure

3.1 Frame preparation and patterning parameters

If you are a first-time user of cymatiX® and mimiX labware, please carefully read the protocol ***Frame Preparation and Patterning Parameters***

3.2 Compound reconstitution and hydrogel preparation

For further information and certificates, please see the manufacturer's homepage

https://www.sigmaaldrich.com/CH/en/search/f4883-500mg?focus=products&page=1&perPage=30&sort=relevance&term=f4883-500mg&type=product

https://www.sigmaaldrich.com/CH/en/product/sigma/t4393?context=product

Volumes in table 3.1 are given for individual solutions of 100 µL each. Please note that the cell suspension will be diluted 5 times, thus prepare a 5x cell suspension concentration. A final concentration of $1{\cdot}10^6$ cells per mL has been shown ideal for a variety of experiments; an initial cell suspension of $5{\cdot}10^6$ cells per mL is prepared.

Both proteins will be prepared at a 2x concentrated solution and subsequently mixed at equal volumes. The final fibrinogen concentration will be 2.5 mg·mL^{-1}, the final thrombin concentration 0.25 IU·mL^{-1}. Mixed solutions gel within minutes and cannot be re-used. Only mix the volumes of thrombin and fibrinogen that you immediately need and keep all reagents on ice.

Table 3.1 *Volumes needed for a total volume of 200 µL fibrin, mixed from equal ratios of fibrinogen solution and thrombin-cell-suspension, resulting in a final concentration of 2.5 mg·mL^{-1} fibrin and 0.25 IU·mL^{-1} thrombin.*

	2x working concentrations	amount stock solution (µL)	Cell Suspension 5x (µL)	medium (µL)
fibrinogen	5 mg·mL^{-1}	16.8	-	83.2
thrombin	0.5 IU·mL^{-1}	0.5	40	59.5

- add the respective volume of medium to two Eppendorf tubes. Let cool on ice.
- add fibrinogen stock solution or thrombin stock solution, respectively, to pre-cooled medium.
- add cell suspension to the thrombin working solution
- keep everything on ice until further use
- mix fibrinogen stock solution at equal volumes with thrombin stock solution.

3.3 Patterning

Frequency, acceleration, and duration must be adjusted in response to the intended application and research question. If you are a first-time user of cymatiX® and mimiX labware, please carefully read the protocol ***Frame Preparation and Patterning Parameters***
Depending on the cell concentration, cell size and the chosen crosslinking strength, a pattern will be formed within 10 seconds to 2 minutes. Fibrin gels within 4 minutes, prolonged patterning is thus not possible, unless work is performed at lower temperatures.

Your cell pattern is ready to be cultured and analysed.

4. Appendix

4.1 Examples and Figures

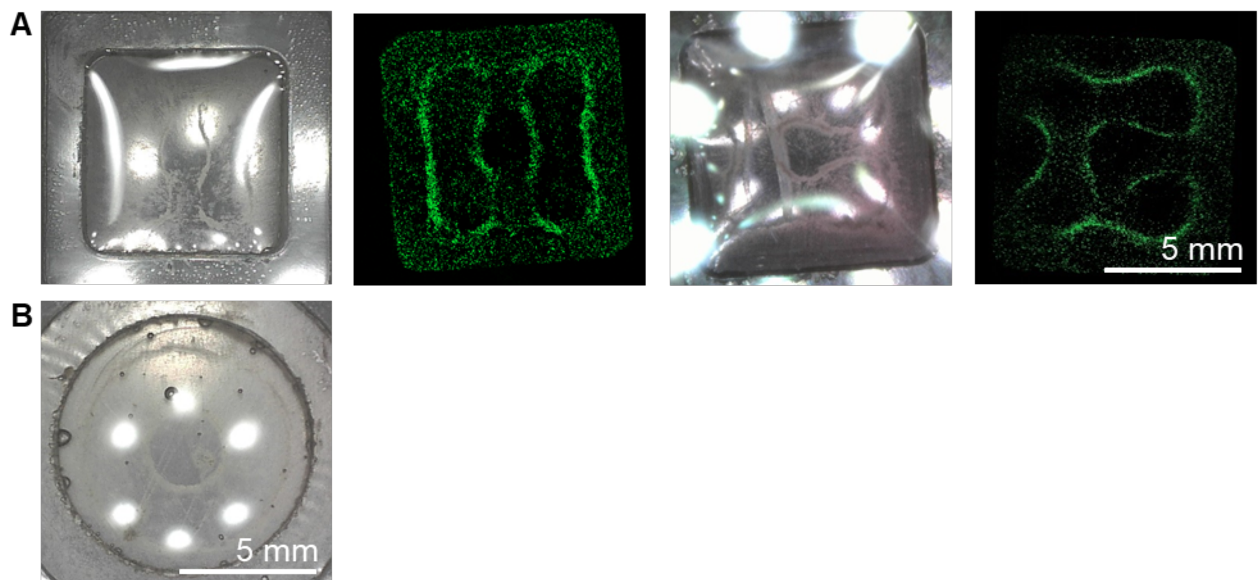

Figure 4.1 *Different examples of endothelial cells patterned in fibrin in A) square or B) round frames.*

4.2 Special Hints

- avoid multiple freeze-thawing cycles of fibrinogen or thrombin. Store in aliquots at -20°C.
- Fibrin and thrombin are natural products. Check gel formation with small amounts prior to each experiment.

4.3 Trouble Shooting Guide

Problem	Solution
fibrin clots immediately	try to always work on ice, it prolongs the time to gelation. Work with fresh aliquots. Gelation kinetics of old (defrosted) samples can change.
fibrin does not clot	Fibrinogen and thrombin are natural products with different percentages of clottable protein fraction and units per mL, with batch to batch variability. Try to increase the fibrinogen concentration at constant thrombin concentration.
No pattern is visible	It is possible that fibrin clotted before the pattern was initiated. Make sure to have everything ready before mixing fibrinogen and thrombin and work on ice whenever possible.
The gel is too liquid at the end	Try to work with a higher fibrinogen concentration. Patterning will be slower at higher concentrations, but the final gel stiffer.