# User Manual: *Patterning Cells in Fibrin*

## Purpose

This user manual outlines the steps for cell patterning in **Fibrin** by use of mimiX labware and cymatiX. It provides guidance for standard cell patterning and highlights the most important DOs and DON'Ts. Please adjust according to your needs and experimental goals.

Additional user manuals needed:

- *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 |
|---|---|---|---|---|
| 1 | Fibrinogen | f4883-500 mg | -20°C | Sigma |
| 2 | Thrombin | T4393-100 UN | -20°C | Sigma |

### 1.2 Additional chemicals and materials needed

- Expanded cells, depending on the experiment
- General cell lab consumables
- mimiX frames
- PBS to wet frames, see user manual *Frame Preparation and Patterning Parameters* for details.
- Sterile tweezers to assemble frames
- NaCl, $CaCl_2$
- Bucket with ice
- Reaction vials
- Petri dishes from Techno Plastic Products AG, Switzerland (www.tpp.ch), 60 mm in diameter, catalogue number 93060

## 2. Preliminary checks and preparation

- If needed, sterilise labware and instruments prior to use
- Calculate the needed amount of chemicals according to table 3.1
- Start cymatiX and perform the initialisation

**Reconstitute Thrombin in sterile filtered 1.1 % (w/v) NaCl in 2 mM $CaCl_2$ at 100 IU per mL**
1 NIHunit = 1.15 IU
With the lot number, the NIH unit for each vial can be checked on the Sigma homepage and the powder reconstituted accordingly. Aliquot and store at -20°C.

**Reconstitute Fibrinogen in sterile filtered 1.1 % NaCl**
Prepare stock solutions of 30 $mg \cdot mL^{-1}$ 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 user manual *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 by a factor of 5, thus prepare a 5x concentrated cell suspension. For example, a final density of $1 \cdot 10^6$ cells per mL has been successfully used for a variety of experiments; accordingly, an initial cell suspension of $5 \cdot 10^6$ cells per mL was 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 $mg \cdot mL^{-1}$, the final thrombin concentration 0.25 $IU \cdot mL^{-1}$. 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 volumes of fibrinogen solution and thrombin-cell-suspension, resulting in a final concentration of 2.5 $mg \cdot mL^{-1}$ fibrin and 0.25 $IU \cdot mL^{-1}$ thrombin.*

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

- Add the respective volume of medium to two reaction vials. 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

Before patterning, please carefully read the user manual *Frame Preparation and Patterning Parameters* which provides examples of patterning parameters. For your own experiment, frequency, acceleration, and duration of the stimulation must be adjusted to address specific experimental requirements and the intended pattern geometry. Depending on the cell density, cell size, and temperature, patterns will be formed within 10 seconds to 2 minutes. For increased local cell density enhancement (increased pattern fidelity), the stimulation can be applied for 2 to 4 minutes. At room temperature, Fibrin gels within 4 minutes, prolonged patterning is thus not possible, unless work is performed at lower temperatures.

Your cell pattern is now ready to be cultured and analysed.

## 4. Appendix

### 4.1 Examples and Figures

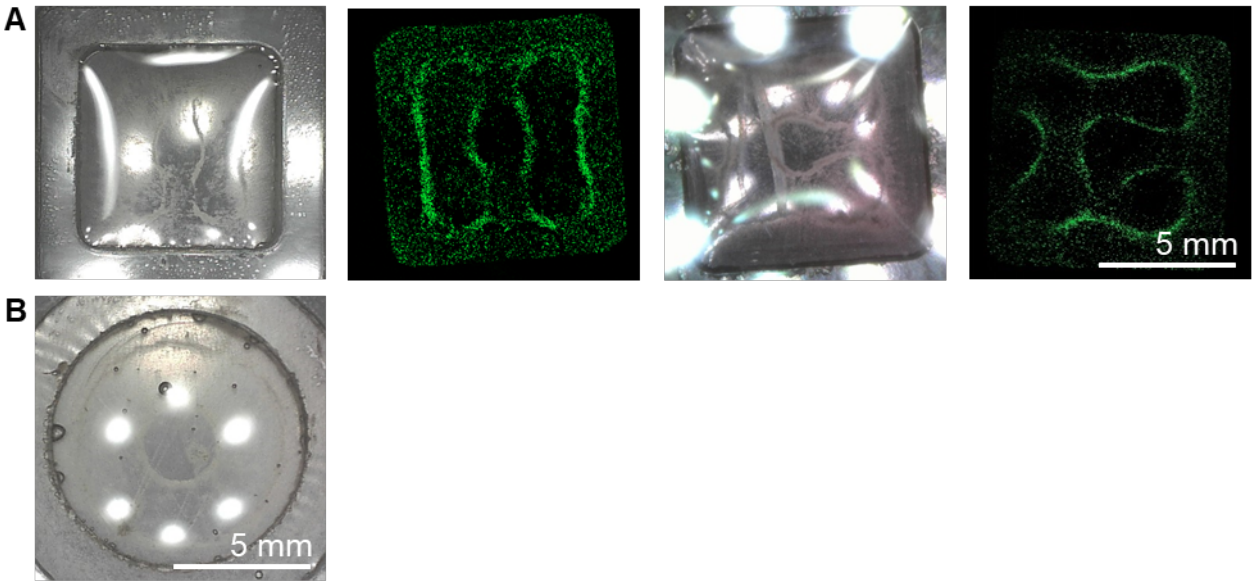


***Figure 4.1*** *Green fluorescent protein expressing human umbilical vein endothelial cells (GFP-HUVEC) were patterned in Fibrin using a series S10 mimiX labware of A) square or B) circular shape. Fluorescence microscopy images were acquired immediately after patterning.*

### 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, at constant thrombin concentration. Patterning will be slower at higher concentrations, but the final gel stiffer. |