User Manual: *Patterning Cells in Rat Tail Collagen*

Purpose

This user manual outlines the steps for cell patterning in **Collagen** 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 Collagen Hydrogels

Rat Tail collagen can be ordered from corning and is provided at concentrations of 3 to 4 mg·mL^{-1} or 8 to 11 mg·mL^{-1}.

- https://ecatalog.corning.com/life-sciences/b2c/US/en/Surfaces/Extracellular-Matrices-ECMs/Corning%C2%AE-Collagen/p/354236
- https://ecatalog.corning.com/life-sciences/b2c/US/en/Surfaces/Extracellular-Matrices-ECMs/Corning%C2%AE-Collagen/p/354249

Collagen is soluble at an acidic pH and is provided as a highly viscous solution in 0.02 N acetic acid. Gelation is initiated upon pH adjustment and is triggered by an increase in temperature. Once neutralised with NaOH, the gel can form within a few minutes at room temperature. Placed on ice, working solutions can be kept liquid for at least a few hours. Collagen is a natural product and batch to batch variability are common. Please always check the data sheet provided with each delivery and the concentration of the specific batch. Neutralise the product following the manufacturer's instructions. The guidelines provided here were established for an initial collagen concentration of 8.63 mg·mL^{-1}. Dilution factors need to be adapted according to the received collagen vial.

Patterning with cymatiX works optimal at collagen concentrations of 0.5 mg·mL^{-1}. This, however, leads to very soft collagen gels. At higher concentrations of 1 to 3 mg·mL^{-1}, the increased viscosity impairs patterning of single cells. An optimal concentration needs to be established for each individual experiment and is dependent on the cell type, cell size (spheroids or single cells) and the intended research goal.

Table 1.1 *Chemicals needed for collagen hydrogels*

Item #	Name	Catalogue #	Storage	Distributor	Comments
1	Collagen, 3-4 mg·mL^{-1}	354236	4°C	Corning	low concentration
2	Collagen, 8-11 mg·mL^{-1}	354249	4°C		high concentration
3	milliQ water, sterile filtered				
4	10x PBS, sterile filtered				
5	1M NaOH, sterile filtered				
6	1x PBS, sterile filtered				

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
- Bucket with ice
- Reaction vials
- Petri dishes from Techno Plastic Products AG, Switzerland (www.tpp.ch), 60 mm in diameter, catalogue number 93060
- If available, positive displacement pipettes to work with collagen at high concentration

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

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://ecatalog.corning.com/life-sciences/b2c/US/en/Surfaces/Extracellular-Matrices-ECMs/Corning%C2%AE-Collagen/p/corningCollagen

Corning Rat Tail Collagen is provided as a sterile solution. Sterile filter all other solutions, if not provided sterile. Always work on ice to avoid collagen gelation.

Volumes in table 3.2 are given for a S10 frame of 80 µL. Due to the high viscosity of collagen and to avoid pipetting of small volumes, a working solution of 3 mg·mL^{-1} is prepared first. This solution can be stored at 4°C for at least a few hours.

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.

Table 3.1 *Volumes needed to prepare a 3 mg·mL^{-1} working solution starting from an initial concentration of 8.63 mg·mL^{-1}.*

from stock solution (µL)	1M NaOH (µL)	10x PBS (µL)	milliQ water (µL)
174	4	50	272

- Add milliQ water to a reaction vial (Eppendorf tube or similar)
- Add 10x PBS and mix well
- Add 1M NaOH, wear eye protection
- Place the mix on ice until cold
- Add collagen and mix well
- Keep collagen working solution on ice at all times

Table 3.2 *Protocol to prepare 85 µL collagen hydrogel to pattern cells in a S10 frame of 0.5 mg·mL^{-1} or 1.0 mg·mL^{-1} final collagen concentration. Volumes of the respective component are given in µL.*

	Final Collagen Concentration	
Component	0.5 mg·mL^{-1}	1.0 mg·mL^{-1}
Collagen working solution (µL)	14	28
1x PBS (µL)	54	40
Cell suspension, 5x (µL)	17	17

- Add PBS to a reaction vial (Eppendorf tube or similar)
- Add collagen working solution and mix well
- Add cell suspension and mix well
- Keep on ice until patterning

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, or collagen concentration, 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 or longer.

After switching off the sound, let collagen solidify on the cymatiX for another 5 to 10 minutes before gently placing the petri dish in a humidified cell culture incubator. After another 20 to 30 minutes medium can be added. For a 60 mm petri dish, 6 mL medium are sufficient to cover the sample. Gently add the medium along the side of the petri dish. It is important that the gel is fully formed prior to medium addition. This can take longer than 30 minutes.

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

4. Appendix

4.1 Examples and Figures

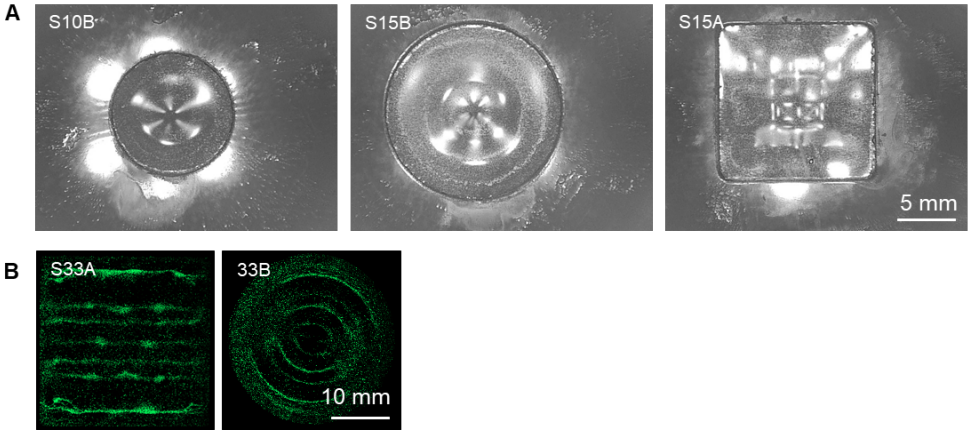

Figure 1 A) *Cells were patterned in a collagen hydrogel at 0.5 mg·mL^{-1} using mimiX labware of series S10 or S15, circular or square.* ***B)*** *Green fluorescent protein expressing human umbilical vein endothelial cells (GFP-HUVEC) were patterned in a collagen hydrogel of 0.5 mg·mL^{-1} in mimiX frames of series S33, square or circular, respectively. Images were acquired immediately after patterning.*

4.2 Special Hints

- For a prolonged patterning experience and time to gelation, the petri dish with the frame can be cooled (placed in a freezer) prior to use.
- For easier pipetting, tips can also be cooled prior to use.
- Collagen concentrations and accordingly the volume of NaOH needed for neutralisation differ from batch to batch. Check carefully prior to mixing.

4.3 Trouble Shooting Guide

Problem	Solution
Collagen gels immediately	Make sure to always work with collagen solutions that were stored on ice. At 0.5 mg·mL^{-1} gelation should not occur immediately, at higher concentrations, it is possible that the gel is highly viscous.
No collagen gel is formed	Collagen is a natural product with high batch to batch variability. Check that the used amount was adjusted in response to the initial collagen concentration. Check the pH of all solutions. Gelation is induced by pH adjustment with NaOH.
No pattern is visible	It is possible that collagen gelled before the pattern was initiated. Make sure to have everything ready and work on ice whenever possible.
The gel is too liquid at the end	Try to work with a higher collagen concentration. Patterning will be slower at higher concentrations, but the final gel stiffer.