User Manual: *Frame Preparation and Patterning Parameters*

This protocol outlines the steps for cell patterning in various hydrogels 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 manual needed

- User manual to operate cymatiX
- User manual to pattern cells in hydrogels of your choice

---

1. Introduction

The steps described here apply to all experiments with mimiX labware and cymatiX, regardless of the hydrogel used and the final application.

Table 1.1 *Chemicals and materials needed*

Item #	Name	Catalogue #	Storage	Distributor	Comments
1	mimiX labware		RT	mimiX	see webshop for different types
2	"the tool"		RT	mimiX	tool to place the frames
3	TPP Petri dishes, 60 mm	93060	RT	TPP	
4	Dentistry Tool		RT	mimiX	tool to remove the frames

1.2 Additional chemicals and materials needed

- Expanded cells, depending on the experiment
- General cell culture consumables
- Hydrogel of choice, depending on the experiment
- PBS
- Sterile tweezers

---

2. Preliminary checks and preparation

- Familiarise yourself with cymatiX and carefully follow the user manual to operate the device
- If needed, sterilise labware and instruments prior to use
- Select the labware size and shape according to your experiment, calculate the appropriate volumes needed for your experiment, including technical repeats and controls

---

3. Procedure

3.1 Frame preparation

- If frames were not received sterile, place frames (double adhesive tape facing upwards) onto a clean surface (e.g. in a large petri dish) and place under the UV in a laminar flow for 30 minutes. With sterile tweezers, turn the frames and place them into a new sterile petri dish. Place under UV for an additional 30 minutes. With sterile tweezers, carefully remove the protective foil from the double adhesive tape, place frames into sterile TPP petri dishes.

- Wash the frame in the dish with 5 mL sterile PBS. Rinse the frame several times with the same solution to make sure no dust particles remain present within the frame. Any dust particles will impair patterning. Aspirate PBS.

- Pre-wet frames with cell culture media; 150 µL for a S10 frame, 1 mm in thickness. Make sure to completely wet the side walls of the frame. A volume of 150 µL is larger than the theoretical volume of the frame. This is intended to completely wet the cut-out, including the top. Let the

medium incubate for 15 minutes or more. Meanwhile, the cell suspension and hydrogel formulations can be prepared. Aspirate the medium just before cell patterning.

- The frame is now ready to be used with cymatiX. Do not let the frame completely dry, a small film of medium will help dispensing the cell-hydrogel suspension properly.

- It is not recommended to incubate frames in Ethanol. The double adhesive tape will be impaired after prolonged incubation in 70% Ethanol and is released immediately if rinsed in 100% Ethanol. It is not recommended to autoclave the frames.

3.2 Patterning

To pattern cells in any hydrogel, use the volumes indicated in Table 3.1 as a guideline. When adding the suspension, start by wetting the walls of the cut-out, followed by gently filling the frame. Use the pipette tip to evenly distribute the suspension within the frame. It is important that the walls are fully wetted (see figure 4.2 and 4.3 below). Gently tilt the frame to check homogeneous dispersion and use a pipette tip to adjust if needed. Close the petri dish and place it gently on the cymatiX platform. Fix with the provided levers and start patterning.

Frequency, acceleration, and duration must be adjusted in response to the intended application and research question. We suggest optimising parameters with cells suspended in culture medium prior to working with hydrogels. Depending on the cell concentration, cell size and the chosen hydrogel, a pattern 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 more, depending on the hydrogel. Table 3.1 provides guidance to choose volume and frequency.

Table 3.1 *Frame dimensions and suggested volumes for cell patterning in* ***square*** *frames.*

Frame	Thickness (mm)	Edge Length (mm)	Cat. #	Theoretical volume (µL)	Volume for patterning (µL)	Frequency (Hz)
S6	0.5	5.7	S06A0500A1	16.1	15-20	
	1.0	5.7	S06A1000A1	32.2	28, 32	40
S10	0.5	9.4	S10A0500A1	44.1	70	
	1.0	9.4	S10A1000A1	88.2	80	60, 70
S15	0.5	13.6	S15A0500A1	93.1	130	
	1.0	13.6	S15A1000A1	186.3	168, 190	60, 50
S21	0.5	18.6	S21A0500A1	173.2	170-190	
	1.0	18.6	S21A1000A1	346.4	311	40
S33	0.5	30.0	S33A0500A1	451.3	450-470	
	1.0	30.0	S33A1000A1	902.6	990	35

Table 3.2 *Frame dimensions and suggested volumes for cell patterning in* ***round*** *frames.*

Frame	Thickness (mm)	Diameter	Cat. #	Theoretical volume (µL)	Volume for patterning (µL)	Frequency (Hz)
S6	0.5	6.4	S06B0500A1	16.1	15-20	
	1.0	6.4	S06B1000A1	32.2	28, 32	40
S10	0.5	10.6	S10B0500A1	44.1	70	
	1.0	10.6	S10B1000A1	88.2	80	60
S15	0.5	15.4	S15B0500A1	93.1	130	
	1.0	15.4	S15B1000A1	186.3	168, 190	60
S21	0.5	21.00	S21B0500A1	173.2	170-190	
	1.0	21.00	S21B1000A1	346.4	276	40
S33	0.5	33.9	S33B0500A1	451.3	450-470	
	1.0	33.9	S33B1000A1	902.6	722, 812, 902	40, 50

3.2 Suggested procedure to evaluate parameters

Hydrogel volumes to pattern cells in different frames deviates from the theoretical volume and strongly depends on the chosen hydrogel and cell type. The values given in table 3.1 and 3.2 proved successful for patterning mammalian cells in hydrogels of the mimiX biomaterial portfolio. By tuning frequency and acceleration, the resulting pattern can be controlled. As a starting point, we suggest a frequency of 60 Hz and an acceleration of 2×g. For parameter evaluation, the following procedure leads to a successful patterning experience:

- Work with fixed cells (protocol below) in cell culture medium or PBS
- Start with a volume larger than the theoretical volume, a frequency of 60 Hz and an acceleration of 2×g. Apply sound for at least 30 seconds before changing any parameter. If you observe streaming or no cell movement, adjust the frequency and acceleration in small steps of 5 units. After each change, wait a few minutes until stable waves are formed. If no patterns are formed, subsequently remove 10 µL cell suspension and repeat parameter adjustment.
- Once parameters were evaluated with fixed cells in cell culture medium, repeat with live cells in cell culture medium.
- Parameters can now be translated to cells in the hydrogel of your choice. Small adjustments might be needed with respect to frequency and acceleration.

3.2.1. Protocol to fix cells for patterning

(1) Expand cells of your choice; 5×10^6 cells is a good number to start with
(2) Trypsinise cells and centrifuge at 250×g or according to your own protocol
(3) Resuspend cells in 10 mL PBS
(4) Centrifuge at 250×g, aspirate supernatant
(5) Gently suspend cells in 4% Formaldehyde and incubate for one hour. (Formaldehyde is toxic, work with adequate PPE and in a chemical hood)
(6) Centrifuge at 250×g, remove supernatant and dispose of in the chemical waste
(7) Resuspend in PBS and centrifuge at 250×g, remove supernatant and dispose of in the chemical waste
(8) Repeat this step three times to wash cells and remove traces of Formaldehyde
(9) Count cells and prepare a cell suspension of 5×10^6 cells per mL in PBS
(10) Fixed cells can be stored at 4°C for further use
(11) Prepare a cell suspension of 1×10^6 cells in PBS for patterning.

3.3 Examples and Figures

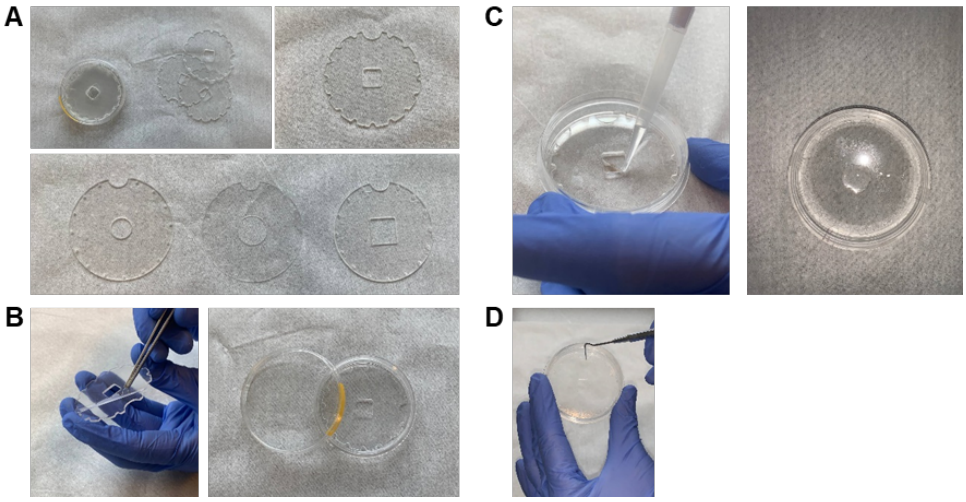

Figure 3.1 A) *Different geometries can be ordered through the online shop. Current models include circular and square frames at different sizes.* ***B)*** *The protective foil is removed with tweezers; the frame is then placed into a petri dish. Press firmly to glue properly.* ***C)*** *Complete wetting of the cut-out is important. Make sure to also wet the surface around the cut-out.* ***D)*** *Upon sample harvesting, frames can be removed with the provided dentistry tool.*

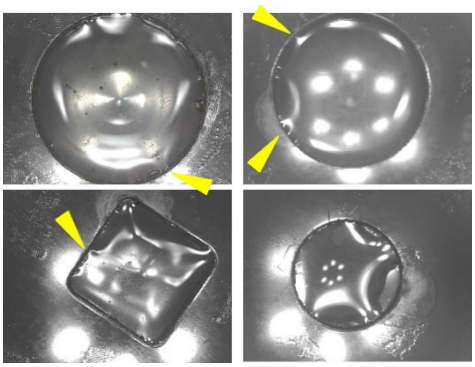

Figure 3.2 *If frames are not wetted carefully, the cell-hydrogel suspension cannot be dispersed adequately within the cut-out. Patterning will not be successful. Yellow arrows point towards regions where the cell suspension is not in good contact with the frame wall. Lower right: A frame without pre-wetting (mimix frames S10, images acquired at different magnifications).*

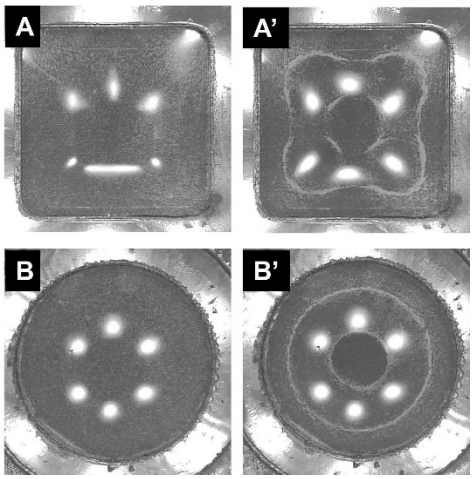

Figure 3.3 *With adequate wetting prior to patterning, the cell suspension is properly dispersed within the frame and patterning will be successful.* ***A*** *and* ***B****: cell suspension before applying sound,* ***A'*** *and* ***B'****: cell patterned formed with sound (mimiX frames S10).*