

User Manual to Pattern Cells in the Hydrogel KYTO

Purpose

This protocol outlines the steps for successful cell patterning in the hydrogel **KYTO** 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 questions.

Additional Protocols needed:

General Protocols

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

1. Description of KYTO

KYTO is a chemically defined, synthetic hydrogel that bears biologically active sites. The main components of this hydrogel are SG-Dextran and CD-link crosslinker. When combined, these two components form stable thioether bonds. The CD-link bears MMP cleavable sites, which allows for potential cell migration. At a crosslinking strength of 2.0 mmol·L⁻¹, a pH of 7.2, and at room temperature, an increase in viscosity is apparent after 2 minutes, complete gelation is achieved within 10 minutes. Crosslinking strength and final mechanical properties can be tuned by varying the SG-Dextran and CD-link concentration. For a prolonged patterning experience, gelation kinetics can be adjusted by changing the pH (optional product 10x CB buffer at pH 5.5). The hydrogel can also be prepared with a thiol-functionalised RGD peptide that covalently binds to the SG-Dextran.

1.1 Chemicals and materials Provided with the mimiX-hydrogel-kit

Item #	Name	Catalogue #	Storage	Distributor	Comments
1	Water	XX	4°C	mimiX	
2	10x CB, pH 7.2	XX	-20°C	mimiX	
3	SG-Dextran	XX	-80°C	mimiX	
4	CD-link	XX	-80°C	mimiX	
5	RGD-SH	XX	-80°C	mimiX	
6	10x CB, pH 5.5	XX	-20°C	mimiX	optional, to tune gelation kinetics

1.2 Additional chemicals and materials needed but not supplied

- Expanded cells, depending on the experiment
- General cell lab consumables
- PBS, optional antibiotics
- sterile tweezers
- bucket with ice
- reaction vials, e.g. Eppendorf tubes.
- mimiX frames (<https://www.mimixbio.com/shop>)
- TPP petri dishes, 60 mm in diameter, catalogue #93060

2. Preliminary checks and preparation

- *Initiate the cymatiX® and run the automatic test*
- *Calibrate all necessary items in accordance with the user manual*

- *If needed, sterilise labware and instruments beforehand.*
- Calculate the needed amount of chemicals according to table 3.1

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***

3.1 Compound reconstitution and hydrogel preparation

Reconstitute the lyophilised powder according to the description provided with your order. In brief, centrifuge the vial with CD-link and add 188 μL water per vial to reach a final concentration of 20 $\text{mmol}\cdot\text{L}^{-1}$ thiol groups. Vortex the vial and incubate for 5 minutes. Vortex and centrifuge again. CD-link is now ready to use. Prepare sterile aliquots of SG-Dextran, 10x CB buffer and PEG-link to avoid repeated freeze-thawing cycles.

To reconstitute RGD-SH, briefly centrifuge the vial and add 48 μL water per vial to receive a final concentration of 20 $\text{mmol}\cdot\text{L}^{-1}$ thiol groups. Incubate for 5 minutes, vortex and centrifuge again. Aliquot to avoid repeated freeze-thawing cycles.

Always work on ice and close the lids immediately after to avoid oxidation of the thiol groups. Do not place the 10x CB buffer on ice after defrosting, precipitates will form. Volumes in table 3.1 are given for a gel crosslinking density of 2.0 $\text{mmol}\cdot\text{L}^{-1}$ and for a S10 frame of 80 μL . 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.

Optional, to prolong time to gelation: Prepare 10x CB buffer at pH 6.9 by mixing 10x CB buffer pH 7.2 with 10x CB buffer pH 5.5 at an 80 to 20 ratio: For a volume of 7.5 μL , mix 6 μL pH 7.2 with 1.5 μL pH 5.5. Use this premixed buffer for further experiments.

Table 3.1 Volumes needed to prepare 85 μL KYTO hydrogel at a crosslinking strength of 2.0 $\text{mmol}\cdot\text{L}^{-1}$

Component	with RGD volume in μL	without RGD volume in μL
water	43.5	47.0
10x CB	6.8	6.8
SG-Dextran	7.1	5.7
RGD-SH	2.1	0
Cell Suspension, 5x	17.0	17.0
CD-link	8.5	8.5

- Mix water, 10x CB buffer (or premixed buffer at pH 6.9) and SG-Dextran
- add RGD-SH if needed and let it incubate for 20 minutes.
- add cell suspension
- pipette up and down well
- when ready to pattern, add the CD-link.

If multiple frames are used, a master mix consisting of water, 10x CB, SG-dextran, RGD-SH and cells can be prepared. For each S10 frame, use 76.5 μL master mix and add 8.5 μL CD-link for a final volume of 85 μL .

3.2 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. For increased local cell density enhancement (more defined pattern), the stimulation can be applied for 2 to 4 minutes.

After switching off the sound, let the hydrogel solidify on the cymatiX® platform for another 2 to 3 minutes before gently placing it in a humidified cell culture incubator. Let it gel for another 15 to 20 minutes prior to medium addition. For a 60 mm petri dish, 6 mL 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.

Your cell pattern is ready to be cultured and analysed.

4. Appendix

4.1 Examples and Figures

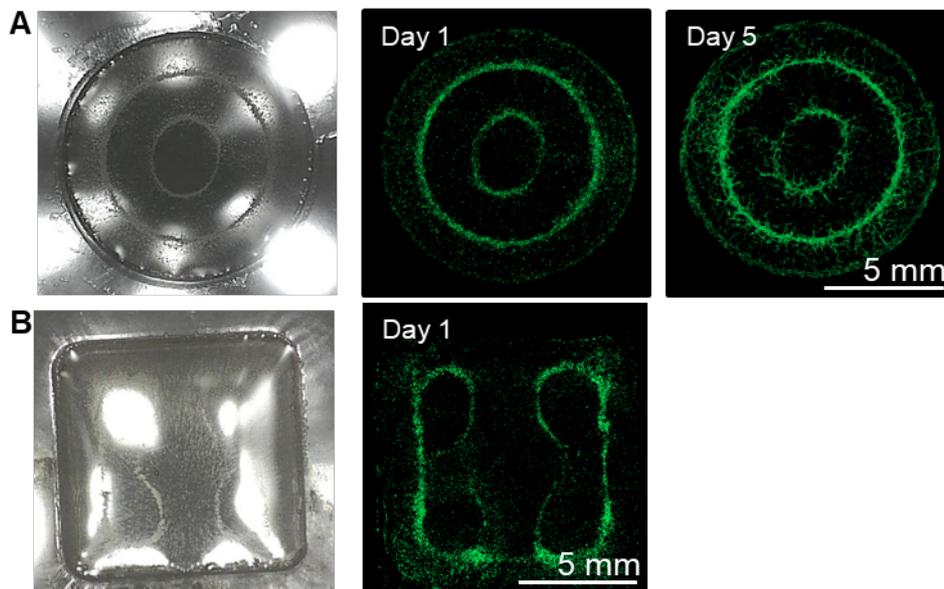


Figure 4.1 Endothelial cells were patterned in KYTO Hydrogel in mimiX labware of A) circular or B) square shape. Fluorescent microscopy images of cells 24 hours or 5 days after patterning. SG++ gels provide an excellent environment for endothelial cell adhesion, assembly, and spreading. The hydrogel was reconstituted at $2.0 \text{ mmol}\cdot\text{L}^{-1}$ and a pH of 6.9.

4.2 Special Hints

no special hints for this gel.

4.3 Trouble Shooting Guide

to be defined ones we are aware of the troubles the customers have