User Manual to Pattern Cells in the Hydrogel BAKU

**Purpose**

This protocol outlines the steps for successful cell patterning in the hydrogel BAKU 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 BAKU

BAKU is a chemically defined, synthetic hydrogel that bears biologically active sites. The main components of this hydrogel are SG-Dextran and PEG-link crosslinker. When combined, these two components form stable thioether bonds. 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 ~16 minutes, complete gelation is achieved after 30 minutes when placed at 37°C. Crosslinking strength and final mechanical properties can be tuned by varying the SG-Dextran and PEG-link concentration. The long time to gelation allows to exploit different patterning parameters or continuous stimulation over 15 minutes to tightly assembly cells. If sound is only applied for a few seconds or minutes, the gel can let to pre-gel for 10 minutes prior to patterning. Otherwise, cells risk to sink to the bottom in the non-viscous gel.

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

<table>
<thead>
<tr>
<th>Item #</th>
<th>Name</th>
<th>Catalogue #</th>
<th>Storage</th>
<th>Distributor</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water</td>
<td>XX</td>
<td>4°C</td>
<td>mimiX</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10x CB, pH 7.2</td>
<td>XX</td>
<td>-20°C or -80°C</td>
<td>mimiX</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>SG-Dextran</td>
<td>XX</td>
<td>-80°C</td>
<td>mimiX</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PEG-link</td>
<td>XX</td>
<td>-80°C</td>
<td>mimiX</td>
<td></td>
</tr>
</tbody>
</table>
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 lyophylised powder according to the description provided with your order. In brief, centrifuge the vial with PEG-link and add 188 µL water per vial to reach a final concentration of 20 mmol L⁻¹ thiol groups. Vortex the vial and incubate for 5 minutes. Vortex and centrifuge again. PEG-link is now ready to use. Prepare sterile aliquots of SG-Dextran, 10x CB buffer and PEG-link 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 mmol-L⁻¹ and for an S1O 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 \(1 \times 10^6\) cells per mL has been shown ideal for a variety of experiments; an initial cell suspension of \(5 \times 10^6\) cells per mL is prepared.

Table 3.1 Volumes needed to prepare 85 µL SG starter hydrogel at a crosslinking strength of 2.0 mmol L⁻¹

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume in µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>47.0</td>
</tr>
<tr>
<td>10x CB, pH 7.2</td>
<td>6.8</td>
</tr>
<tr>
<td>SG-Dextran</td>
<td>5.7</td>
</tr>
<tr>
<td>Cell Suspension, 5x</td>
<td>17.0</td>
</tr>
<tr>
<td>PEG-link</td>
<td>8.5</td>
</tr>
</tbody>
</table>

- Mix water, 10x CB buffer and SG-Dextran
- add cell suspension
- pipette up and down well
- when ready to pattern, add the PEG-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, mix 76.5 µL master mix with 8.5 µL PEG-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 up to 15 minutes until the viscosity increases.

After switching off the sound, let the hydrogel solidify on the cymatIX® platform for another 5 to 10 minutes before gently placing it in a humidified cell culture incubator. Let it gel for another 30 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

![Image of cell pattern](image1)

#### 4.2 Special Hints

-no special hints for this gel.