PRIMO PROTOCOL: HYDROGEL PHOTOPOLYMERIZATION

This protocol is a guideline and you might need to optimize it for each different application.

Protocol for the use of 4 ARM PEG-ACRL, MW 10,000 hydrogel (Laysan Bio, Inc.)

Equipment

- Inverted microscope
- Motorized XY-stage
- Camera
- PRIMO®
- Control software LEONARDO
- Computer
- Micropipettes and adequate tips

Reagents

- 4 ARL PEG-ACRL,MW 10,000 hydrogel
- PLPP 1x solution
- Deionized water
- PBS 1x solution pH = 7,4

1. Hydrogel solution preparation

4 ARL PEG-ACRL,MW 10,000 hydrogel

- Dissolve hydrogel at 10% in H_20.
- Add 20 to 50 % of PLPP to the hydrogel solution.
- Wash 5 times with PBS.

2. Illumination

The height of the hydrogel will depend on the height of illuminated liquid. To control it is possible to place the hydrogel solution in a chamber of controlled height.

- Place the solution in the PDMS chamber.
- Focus in your well.
- Illuminate the hydrogel with your UV pattern (typical UV dose: 300-900mJ.mm^{-2}).

If working with NIS plugin insolate for 10 to 30s

- Rinse 5 times with PBS.

You can control the reticulation of the gel by changing the hydrogel concentration, the PLPP percentage and the UV dose.

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Protocol for the use of Collagen I – Methacrylate (Photocol kit, Methacrylated Type I Collagen for crosslinkable hydrogels, cat n°5201-1EA, lot n°7519, Advanced Biomatrix).

Equipment

- Inverted microscope
- Motorized XY-stage
- Camera
- PRIMO®
- Control software LEONARDO
- Computer
- Micropipettes and adequate tips

Reagents

- Collagen I – Methacrylate (Advanced Biomatrix)
- PLPP 1x solution
- Acetic acid
- PBS 1x solution pH = 7,4

3. Hydrogel solution preparation

Collagen I – Methacrylate (Advanced Biomatrix)

- Dissolve hydrogel at 8mg/mL in acetic acid.
- Add 50 % of PLPP to the hydrogel solution.
- Micropipettes and adequate tips
- Wash 5 times with PBS.

4. Illumination

The height of the hydrogel will depend on the height of illuminated liquid. To control it is possible to place the hydrogel solution in a chamber of controlled height.

- Place the solution in the PDMS chamber.
- Focus in your well.
- Illuminate the hydrogel with your UV pattern typical UV dose: 300-600mJ.mm^{-2}).
- Rinse 5 times with PBS.

You can control the reticulation of the gel by changing the hydrogel concentration, the PLPP percentage and the illumination time. **/!\ Always work on ice.**

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Protocol for the use of 4 ARM PEG-ACRL, MW 10,000 hydrogel (Laysan Bio, Inc.) AND Collagen I – Methacrylate (Photocol kit, Methacrylated Type I Collagen for crosslinkable hydrogels, cat n°5201-1EA, lot n°7519, Advanced Biomatrix).

Equipment

- Inverted microscope
- Motorized XY-stage
- Camera
- PRIMO®
- Control software LEONARDO
- Computer
- Micropipettes and adequate tips

Reagents

- 4 ARL PEG-ACRL,MW 10,000 hydrogel
- Collagen I – Methacrylate
- PLPP 1x solution
- Deionized water
- Acetic acid
- PBS 1x solution pH = 7,4

5. Hydrogel solution preparation

4 ARL PEG-ACRL,MW 10,000 hydrogel

- Dissolve hydrogel at 10% in H20.
- Add 20 to 50 % of PLPP to the hydrogel solution.
- Micropipettes and adequate tips
- Wash 5 times with PBS.

Collagen I – Methacrylate (Advanced Biomatrix)

- Dissolve hydrogel at 8mg/mL in acetic acid.
- Add 50 % of PLPP to the hydrogel solution.
- Micropipettes and adequate tips
- Wash 5 times with PBS.

For the mix of the both hydrogels:

- Optimal concentration: 80% Collagen I – Methacrylate (8mg/mL) + 20% 4arm-PEG-Acrylate (10%)
- Add 50% of PLPP to the hydrogel mix

6. Illumination

The height of the hydrogel will depend on the height of illuminated liquid. To control it is possible to place the hydrogel solution in a chamber of controlled height.

- Place the solution in the PDMS chamber.

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- Focus in your well.
- Illuminate the hydrogel with your UV pattern (typical UV dose: 300-600mJ.mm^{-2}).
- Rinse 5 times with PBS.

You can control the reticulation of the gel by changing the hydrogel concentration, the PLPP percentage and the illumination time. **/!\ Always work on ice and prepare the mix extemporaneously.**

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