# Preparation of *3-D Life* Hyaluronic Acid (HA) Hydrogels

## 1. Introductory Notes

- *3-D Life* Hyaluronic Acid (HA) Hydrogels are biochemically defined hydrogels that can be applied for three-dimensional cultivation, cultivation of cells on top of gel or co-cultivation of many cell types.
- Ease of use and complete control of bio-molecular modifications and gel stiffness allow for an extensive variety of cell culture applications.
- The hydrogel is formed by cross linking of thiol-reactive dextran or polyvinyl alcohol (PVA) with thiol-functionalized hyaluronic acid. The presence of hyaluronic acid allows the spreading and migration of most cell types, if cell adhesion molecules (for example RGD Peptide, Cat. No. 09-P-001 or GFOGER-3 Peptide, Cat. No. P12-1) are present in the gel and the cells display the appropriate receptors.
- If prepared with thiol-reactive dextran, *3-D Life* HA Hydrogel can be dissolved by the addition of *3-D Life* Dextranase (Cat. No. D10-1) to recover chemically fixed or live cells.

## 2. Protocol

The following protocol describes the preparation of *3-D Life* HA Hydrogels for 3-D cell culture with and without modification of the gel matrix with the cell adhesion peptides *3-D Life* RGD and/or GFOGER-3. Please read the full protocol before you start preparing a gel.

### Reagents and materials

| Hydrogel Kits | Catalog Number |
|---|---|
| *3-D Life* Dextran-HA Hydrogel SG | G95-1 |
| *3-D Life* PVA-HA Hydrogel SG | G85-1 |
| **Peptides** | |
| *3-D Life* RGD Peptide | 09-P-001 or P10-3 |
| *3-D Life* GFOGER-3 Peptide | P12-1 or P12-3 |
| **Related Products** | |
| *3-D Life* Dextranase | D10-1 |
| *3-D Life* 10x CB pH 5.5 | B10-3 |

**Reagents and materials not included in the *3-D Life* products:**

Cell culture medium, cell culture plate, reaction tubes, pipet tips, micropipets, serological pipets.

## Preparations

**Hydrogel reagents:**

- Dissolve the HyLink lyophilisate of the *3-D Life* PVA-HA or *3-D Life* Dextran-HA Hydrogel kits according to the instructions in the accompanying Product Data Sheet.
- If hydrogel reagents are frozen, thaw all reagents at room temperature. Make sure that salts in the 10x CB (pH 7.2) buffer are completely dissolved. If necessary, put the buffer vial in a 37°C water bath until all salts are dissolved. Do not put 10x CB (pH 7.2) on ice, this may cause the salts to crystallize.
- If lyophilized peptides are used, reconstitute the peptide according to the instructions on the accompanying Product Data Sheet.

**Note:** Do not expose thiol-containing reagents (RGD Peptide, GFOGER-3 Peptide, HyLink) to air and room temperature longer than necessary to avoid oxidation of the thiol groups. Close cap after each use.

**Cell suspension:**

Prepare a stock cell suspension or any other biological sample of your choice in culture medium, PBS or in any other physiological solution. When preparing this sample, consider that the volume of this sample will be no more than 1/5 of the final gel volume. Accordingly, the cell density in the gel will be only 1/5 of the stock cell suspension.

## Experimental procedure

The following protocol describes the preparation of a soft hydrogel (cross linking strength of 1.2 mmol/L) with the option of modification with 0.5 mmol/L RGD and/or 1.2 mg/ml GFOGER-3 Peptide. The volumes of gel reagents required for 100 µl of gel are listed in Table 1.

*Table 1: Reagent volumes for 100 µl of gel using SG-Dextran or SG-PVA polymer to be cross-linked with 1.2 mmol/L SH groups of the cross-linker HyLink (1.2 mmol/L cross linking strength) with the option of modification with 0.5 mmol/L RGD Peptide and/or 1.2 mg/ml GFOGER Peptide.*

| Reagents | Volumes for 100 µl gel (µl) | | | |
|---|---|---|---|---|
| | w/o Peptide | with RGD Peptide | With GFOGER-3 Peptide | with RGD and GFOGER-3 Peptide |
| Water | 56 | 51.8 | 48.3 | 44.2 |
| 10x CB, pH 7.2 | 8 | 8 | 8 | 8 |
| SG-Dextran or SG-PVA (30 mmol/L SH-reactive groups) | 4 | 5.7 | 5.7 | 7.3 |
| RGD Peptide (20 mmol/L SH groups) | - | 2.5 | - | 2.5 |
| GFOGER-3 Peptide (20 mg/mL) | - | - | 6 | 6 |
| Cell suspension | 20 | 20 | 20 | 20 |
| HyLink (10 mmol/L SH groups) | 12 | 12 | 12 | 12 |
| Total | 100 | 100 | 100 | 100 |

If not indicated otherwise, all steps below are performed in a sterile hood at room temperature:

1. Combine Water, 10x CB (pH 7.2) and the SG-Polymer of your choice (SG-Dextran or SG-PVA) in a reaction tube. Mix well.
2. If peptides are used (otherwise continue with step 3):

   Add the peptide(s) and mix immediately to ensure homogeneous modification of the SG-Polymer with the peptide(s). Incubate sample for 20 min to allow the peptide(s) to attach to the SG-Polymer.
3. Add the cell suspension.
4. Add the crosslinker HyLink. Immediately mix by pipetting up and down a few times.
5. After addition of the crosslinker incubate the pre-gel solution for up to 10 minutes (SG-PVA) or 12 minutes (SG-Dextran) at room temperature. Do not incubate longer because the solution will begin to solidify and cannot be pipetted anymore. Resuspend cells to ensure that cells will be uniformly distributed later in the gel and transfer the pre-gel solution in a culture dish. Incubate for 25 minutes at room temperature or at 37°C in the incubator to allow the gel to solidify.
6. Make sure that the gel has solidified and carefully add cell culture medium until the gel is covered.
7. Place culture dish in the incubator for cultivation of cells.
8. Renew medium after 1 hour.
9. Change the medium as needed during cultivation of cells.

## 3. Variations of Gel Preparations

Reagent volumes for gel variations described below can easily be calculated using the online calculation tool on www.cellendes.com.

**Preparation of small gel volumes**
If small volumes of gel are prepared (less than 100 µl) only very small volumes of the RGD Peptide stock solution are required. To avoid the pipetting of such small volumes, it is recommended to reduce the concentration of the RGD Peptide stock solution to 3 mmol/L by dilution with water. This increases the volume to be pipetted. The volume of water has to be reduced accordingly.

**Preparation of multiple gels of same composition**
To generate multiple gels of same composition, aliquots of the pre-gel solution are placed in the culture dishes. It is recommended to resuspend cells in the pre-gel solution each time before an aliquot is pipetted to obtain an equal number of cells in each gel.

**Preparation of gels with different concentrations of peptides**
If gels of different concentrations of adhesion peptide are to be prepared, please consult the User Guide or the online calculator for calculating volumes of reagents.

**Note:** If greater concentrations of GFOGER-3 peptide are used, be aware that GFOGER-3 has cross-linking properties. It is recommended to test the gelation speed before starting with the experiment. If higher concentrations of GFOGER-3 are used, the cross linking may interfere with cell spreading and migration because GFOGER-3 is not enzymatically cleavable by cells.

**Preparation of plain gels (without cells) or embedding other specimens**
If no cells are included in the gel, e.g. for encapsulation of tissues or preparation of plain gels, replace the volume of cell suspension with PBS or other physiologically compatible solution of your choice. Alternatively, use the online calculator on www.cellendes.com and keep the component „Cell

Suspension or Similar“ blank or enter "0".

### RGD Peptide replacements for control experiments

Instead of the RGD or GFOGER-3 Peptide, Thioglycerol (Catalog Number T10-3) can be added to a gel. In this case the gel does not provide cell attachment sites and can be used as a control to RGD Peptide-modified gels. Cellendes also offers a scrambled version of RGD Peptide for control experiments (*3-D Life* Scrambled Peptide, Catalog Number 09-P-003).

### Preparation of gels of higher stiffness

Gels of higher stiffness than the gels described in Table 1 can be made by increasing the concentrations of the SG-Polymer (SG-Dextran or SG-PVA) and crosslinker HyLink. For calculating reagent volumes, please consult the User Guide or the calculation tool on www.cellendes.com.

With increasing gel stiffness the time between addition of crosslinker and the beginning of solidification of the gel solution becomes considerably shorter. In Table 2 approximate time periods of the fluid state as well as the time points after which gels are solid enough for the addition of medium are indicated for gels with cross linking strengths of 1.2 mmol/L.

**IMPORTANT:** The times given in table 2 provide a rough guideline only. It is recommended to perform a test run of gel preparation without cells to confirm times of fluid state and time for the gel to solidify before you start your experiment.

*Table 2: Gelation times of hydrogels crosslinked with HyLink at 1.2 cross linking strength at room temperature. GFOGER-3 peptide concentration: 1.2 mg/ml.*

| Cross linking strength | Time after mixing polymer and crosslinker | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SG-Dextran + HyLink | | | | SG-PVA + HyLink | | | |
| | Fluid up to | | Addition of medium after | | Fluid up to | | Addition of medium after | |
| | w/o GFOGER-3 | with GFOGER-3 | w/o GFOGER-3 | with GFOGER-3 | w/o GFOGER-3 | with GFOGER-3 | w/o GFOGER-3 | with GFOGER-3 |
| 1.2 mmol/L | 15 min | 11 min | 25 min | 25 min | 11 min | 10 min | 25 min | 25 min |

### Slowing down gelation by pH reduction for highly crosslinked hydrogels

If hydrogels of higher stiffness than indicated in Table 2 are prepared, the time to keep the pre-gel solution fluid and pipettable can be extended by reducing the pH. Usually, the preparation of *3-D Life* HA Hydrogels is performed at pH 7.2. 10x CB of lower pH can be generated by mixing 10x CB (pH 7.2) with 10x CB (pH 5.5) (Catalog Number B10-3) (Fig. 1). Such a mixture of 10x CB can be used instead of 10xCB (pH 7.2) to slow down the gel formation.

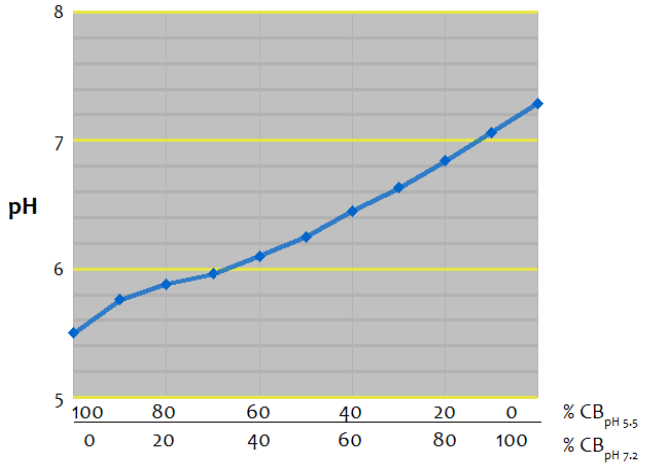


*Figure 1: pH resulting from different mixing ratios of 10x CB pH5.5 and 10x CB pH 7.2.*

## 4. Dissolving SG-Dextran Hydrogels with Dextranase

Live or chemically fixed cells can be recovered from Dextran-HyLink Hydrogels by the enzymatic digestion of the gels with dextranase (Catalog Number D10-1). Dextranase is added to the culture medium or buffer at a 1:20 dilution. For example, a 30 µl gel can be dissolved by adding 300 µl of a 1:20 dilution of dextranase in medium followed by an incubation of 30-60 minutes at 37°C. Gels can be dissolved faster, if they are cut in pieces.

After dissolution of the gel, centrifuge the cell suspension and resuspend the pelleted cells in fresh medium or physiological buffer as required. Repeat this washing procedure twice to more effectively remove remnants of dextranase and dissolved gel components. The removal of dextranase is important when cells are being embedded again in dextran hydrogels for a continued culture. Small traces of dextranase can destabilize the newly set up hydrogel.

For a detailed protocol see Technical Protocol TP-1 on www.cellendes.com.