3-D Cell Culture with *3-D Life* ToGro Hydrogel

1. Introductory Notes

- *3-D Life* ToGro Hydrogel is a chemically defined hydrogel that can be applied for three-dimensional cultivation of many cell types. It has a soft stiffness of approximately 400 Pa (shear modulus). The defined concentration of the RGD cell adhesion peptide (0.4 mmol/l) combined with the cell-degradable crosslinker CD-Link mediates adhesion, spreading and migration of most cells. The gel is formed by the crosslinking of modified dextran carrying the cell adhesion motif RGD (RGD-Dextran) with CD-Link. Once the crosslinker CD-Link is added the gelation speed leaves enough time (approximately four minutes) to conveniently place the reagent mix into a culture dish before the solution solidifies. Due to the pre-defined combination of reagents the setup of the hydrogel is very simple by following this protocol.
- *3-D Life* ToGro Hydrogel can be dissolved by the addition of dextranase (Cat. No. D10-1) to recover chemically fixed or live cells.

2. Protocol

2.1. Embed cells in ToGro Hydrogel

Reagents and materials:

***3-D Life* products:**

3-D Life ToGro Kit (Cat # G94-1)

Optional: *3-D Life* Dextranase (Cat # D10-1)

Additional Reagents and materials:

Cell culture plate

Cell culture medium or similar

Cell suspension

Other material: reaction tubes, pipet tips, micropipets, serological pipets

Preparations:

If not already done for previous gel preparations, reconstitute the RGD-Dextran and CD-Link lyophilisates as described in the *3-D Life* ToGro Product Data sheet.

Note: Do not expose CD-Link to air longer than necessary to avoid oxidation of the thiol groups. Close cap after each use. If CD-Link is used for longer than one hour, keep it on ice or at 4°C.

Experimental procedure:

If not indicated otherwise all steps below are performed in a sterile hood:

1. Prepare a 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 should be 20% of the final volume of the gel (see Table 1). For example, if a hydrogel of 25 µl is prepared, use 5 µl of cell suspension.
2. Combine RGD-Dextran and cell suspension in a reaction tube in volume ratios as indicated in Table 1 and mix gently. Add CD-Link to the mixture and mix again by pipetting up and down a few times.

   Table 1: *Mixing ratios of ToGro Hydrogel reagents*

3-D Life reagents	Volume parts	Examples of hydrogel reagent volumes (µl)		
RGD-Dextran	18	18	36	72
Cell suspension or similar	5	5	10	20
CD-Link	2	2	4	8
Final gel volume	25	25	50	100

3. Place the mixture in a culture dish. Do not wait longer than 4 minutes for the placement of the mixture. After this time the solution begins to solidify and may not be pipettable anymore.
4. Incubate the mixture for 20 minutes at 37°C in the incubator for gel formation. Alternatively, gelation can be performed at room temperature with a slightly longer incubation time.

   Note: Make sure that the gel has hardened before adding culture medium in step 5. Optional: test gel formation by careful inspection with a pipet tip. The tip should not pull out threads of gel when touching and retracting from the gel surface.
5. Once the gel has formed, cover the gel with culture medium.
6. Cover the culture dish and place it in the incubator for cell cultivation.
7. Renew medium after 1 hour.
8. Replace medium during further cultivation as required for proper growth of cells.

2.2. Dissolving ToGro Hydrogels with Dextranase

ToGro Hydrogels containing live or chemically fixed cells can be dissolved by adding dextranase to the culture medium of the hydrogel culture. For example, a 25 µl gel can be dissolved with 300 µl of a 1:20 dilution of dextranase in medium incubated for 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 once or twice to more effectively remove remains of dextranase and dissolved gel components. The removal of dextranase is important when cells are being embedded again in dextran hydrogels to continue culture. If dextranase is not removed completely, it can destabilize the newly set up hydrogel.