Co-culture of tumor and stroma cells in *3-D Life* Hydrogels

1. Introductory Notes

- The *3-D Life* Hydrogel technology and its applications are described in the *3-D Life* Hydrogels User Guide which can be downloaded from www.cellendes.com.
- For the generation of a co-culture of tumor and stroma cells, both cell types are embedded in hydrogels formed by crosslinking of Mal-Dextran with cell-degradable CD-Link. Prior to crosslinking of Mal-Dextran with CD-Link, Mal-Dextran is modified with RGD Peptide.
- The dextran-based gel can be degraded with dextranase which allows the isolation of cells from the gel after culture for further processing.

2. Setup of 3-D co-culture

Reagents and materials:

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

3-D Life Dextran-CD Hydrogel Kit (Cat # G91-1)

3-D Life RGD Peptide (Cat # 09-P-001)

The reagents produce 44 hydrogels each with a volume of 30 µl.

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

Cell culture plate: e.g. 8-chamber slide or multiwell plate, preferably with glass bottom or equivalent for inverse microscopy.

Cell culture medium: DMEM containing 10% fetal bovine serum, 4 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin and DMEM/Ham´s F12 (1:1) containing 10% fetal bovine serum, 2,5 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. The two media are mixed at a ratio of 1:1.

Sterile PBS w/o Ca/Mg (PBS^-)

Human breast cancer cells (MCF-7): cell suspension in PBS^- at a cell density of 6x 10^5 MCF-7 cells/ml

Primary human dermal fibroblasts: cell suspension in PBS^- at a cell density of 2x 10^6 fibroblasts/ml

Other material: Eppendorf tubes, pipet tips, micropipets, serological pipets, ice

Preparations:

- If hydrogel reagents are provided in lyophilized form, dissolve the lyophilisates according to the instructions in the accompanying Data Sheets. If hydrogel reagents are provided frozen, thaw all reagents at room temperature. Make sure that salts in the 10x CB pH 5.5

are completely dissolved. Keep 10x CB pH 5.5 at room temperature during use.

- Place Mal-Dextran on ice.

Note: Do not expose *3-D Life* thiol-containing reagents (RGD Peptide, CD-Link) to air longer than necessary to avoid oxidation of the thiol-groups. Close cap after each use.

Embed cells in hydrogel:

The volumes of gel components for 30 µl gels are listed in Table 1. If several gels with the same composition are generated, a reagent mix (Reagent Mix A) consisting of 10x CB, Water, Mal-Dextran, RGD Peptide and the cell suspension can be prepared in multiples of the indicated volumes (see 3x example in Table 1).

Note: We recommend preparing the reagent mix with one excess volume of each component to avoid a potential shortage of reagent mix in case slight inaccuracies in pipetting occur.

Table 1: *Reagent volumes for 30 µl gels crosslinked with 3 mmol/l maleimide- and SH groups, and modified with 0.5 mmol/l RGD Peptide.*

3-D Life reagents	Final concentrations in the gel	1x (µl)	3x (µl)	
10x CB, pH 5.5	n.a.	2.0	6.0	= Reagent mix A
Water	n.a.	9.2	27.6	
Mal-Dextran (30 mmol/l maleimide groups)	3.5 mmol/l maleimide groups	3.5	10.5	
RGD-Peptide (20 mmol/l SH groups)	0.5 mmol/l SH groups	0.8	2.4	
MCF-7 Cell suspension (6x 10^5 cells/ml)	1x 10^5 cells/ml	5.0	15.0	
Fibroblasts cell suspension (2x 10^6 cells/ml)	3.3x 10^5 cells/ml	5.0	15.0	
CD-Link (20 mmol/l SH groups)	3 mmol/l SH groups	4.5	3x 4.5	
Total		30.0	3x 30.0	

Note: The ratio of tumor cells to fibroblasts in this culture is 1:3.3.

All steps below are performed under a sterile hood:

- Combine 10x CB (pH 5.5), Water and Mal-Dextran in an Eppendorf tube. Mix well.
- Add the RGD Peptide and mix immediately to ensure homogenous modification of the Mal-Dextran with the peptide.
- Incubate sample for 5-10 min at room temperature to allow for the RGD Peptide to attach to the maleimide groups of the dextran polymer.
- Place 4.5 µl of crosslinker (CD-Link) on the surface of a culture dish.

- Add cells to the reaction tube containing the peptide-conjugated Mal-Dextran to complete Reagent mix A.
- In a pipet tip, transfer 25.5 µl of Reagent mix A to the 4.5 µl CD-Link on the dish and quickly mix both by pipetting up and down three times. Avoid the formation of air bubbles. Leave the mix on the surface of the culture dish. Wait for approximately 3 minutes to let the gel form.

  Note: Gel formation starts after a few seconds of mixing. Complete mixing step as fast as possible to avoid gel formation in the pipet tip.
- Optional: test gel formation by careful inspection with a pipet tip.
- Once the gel has formed, add cell culture medium until the gel is covered.
- Use lid to cover the culture dish, then place the dish in the incubator.
- Renew the medium after 1 hour.
- Replace medium every 2-3 days.