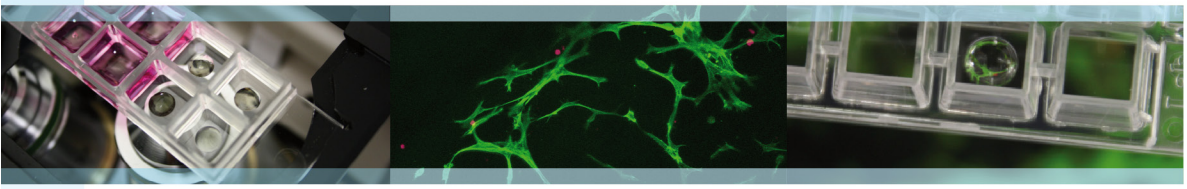

CELLENDES
Cell • Environment • Design

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

The role of stroma in tumor progression

The role of stroma in cancer progression is gaining increased attention in research and drug development (Polyak and Kalluri, 2010; Anton and Glod, 2009). 3-D *in vitro* co-culture of cancer cells and cells of the tumor microenvironment allow the investigation of this important interplay between these cells. Biochemically defined 3-D matrices are crucial to distinguish between effects of matrix components and factors produced by the cells during co-culture.

A tumor-stroma model in *3-D Life* Hydrogels

3-D Life Hydrogels are used to co-culture the human breast cancer cell line MCF-7 with primary human dermal fibroblasts (Figure 1 A-C). MCF-7 cells cultured alone in hydrogels form tumor-like spheroids (Fig. 1 A), whereas human dermal fibroblasts cultures alone in hydrogel appear in an outstretched phenotype typical for dermal fibroblasts *in vivo* (Fig. 1 B). The co-culture of both cell types results in a tumor-stroma model that allows the specific manipulation of the culture with additional factors and the analyses of the effects of this co-culture on each cell type (Fig. 1 C).

References

Polyak K., Kalluri, R. The role of the microenvironment in mammary gland development and cancer. Cold Spring Harb. Perspect. Biol. 2010;2:a003244.

Anton, K, Glod, J. Targeting the tumor stroma in cancer therapy. Curr. Pharm. Biotechnol. 2009;10(2):185-191.

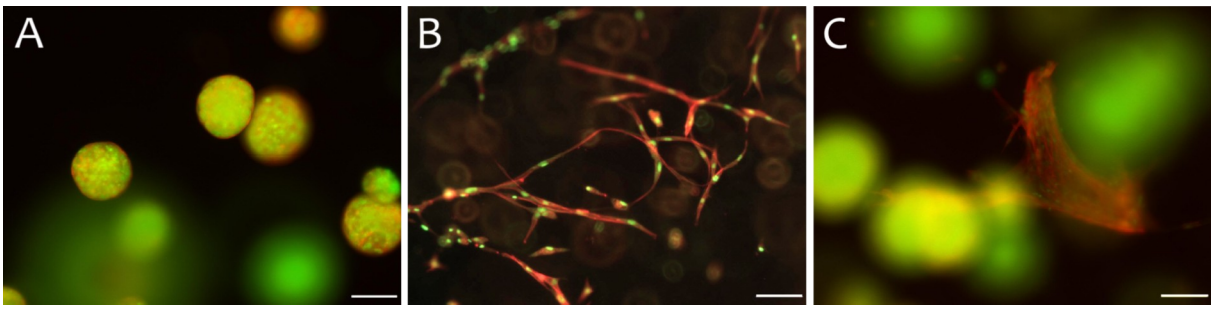

Fig. 1: Epifluorescence microscopy of mono- and co-culture of MCF-7 cancer cells and human primary dermal fibroblasts in 3-D Life Hydrogels. Cells were cultured for 14 days in Dextran gels modified with 0,5 mmol/l RGD peptide as described in Methods. A: MCF-7 cells cultured alone, B: fibroblasts cultured alone, C: co-culture of MCF-7 and fibroblasts. Red: actin cytoskeleton; green: nuclei. Scale bar: 100 µm.

Methods

Cells were cultured in 30 µl *3-D Life* Dextran Hydrogels crosslinked with MMP-cleavable CD-Link at a crosslinking strength of 3 mmol/l maleimide (Maleimide-Dextran) and SH (CD-Link) groups and modified with 0.5 mmol/l RGD peptide. Gels were prepared as described in the *3-D Life* User Guide. 3,000 MCF-7 cells and 10,000 fibroblasts were seeded alone or combined in one gel. Gels were incubated in DMEM (low glucose) containing 4 mmol/l L-Glutamine and DMEM/Ham´s F12 (1:1) containing 2.5 mmol/l L-Glutamine at a ratio of 1:2 supplemented with 10% (v/v) FBS. After 14 days of culture, cells in hydrogels were chemically fixed in 4 % paraformaldehyde in PBS for 1 hour and washed four times for 5 min in PBS. Cells were permeabilized with 0,5 % (v/v) Triton® X-100 in PBS for 10 min and washed three times for 10 min in PBS.

Gels were incubated with 1.7 µg/ml phalloidin-TRITC (Sigma) in PBS for 1.5 hr in the dark and subsequently washed three times for 5 min in PBS. Nuclei were stained by incubation of gels with 17 µmol/l Syto 24 Green® (Invitrogen) for 30 min at room temperature in the dark. Gels were washed three times 5 min with PBS and stored in PBS at 4°C. Cells in the gel were observed by epifluorescence microsopy.

Products used

3-D Life Dextran-CD Hydrogel FG, Cat. No. FG91-1
3-D Life RGD Peptide, Cat. No. 09-P-001

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