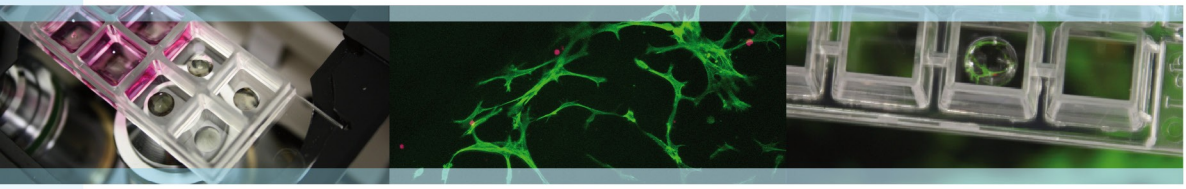

CELLENDES
Cell • Environment • Design

Application Note 2:
Fibroblast Spreading in Cell-Degradable *3-D Life* Hydrogels

Cell spreading requires MMP cleavage and cell adhesion sites

3-D Life Hydrogels consist of a dense network or polymers with small pores at an estimated size of only several nanometers. If cells are supposed to spread and migrate within the gel, the dense network of polymers must be broken up by the cells. *3-D Life* CD-Link is a crosslinker that contains a matrix metalloprotease (MMP) cleavable recognition site which can be cleaved by cells if they produce the corresponding MMPs. As a consequence, the hydrogel network locally loosens up and cells can spread within the gel (illustrated in Fig. 1).

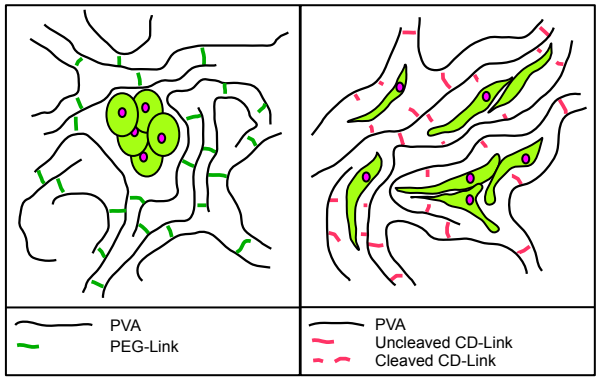

Figure 1: Schematic drawing of cells growing in 3-D Life PVA Hydrogel crosslinked with either 3-D Life PEG-Link (left) or 3-D Life CD-Link (right).

3T3 fibroblasts were grown in PVA hydrogel that was either crosslinked with the undegradable crosslinker *3-D Life* PEG-Link or the MMP cleavable *3-D Life* CD-Link (Fig. 2). Cells grown in hydrogels with CD-Link spread whereas cells grown in gels crosslinked with PEG-Link stay round in tightly packed aggregates. Spreading of fibroblasts in *3-D Life* Hydrogels does also require the presence of adhesion sites, e.g. *the 3-D Life* RGD Peptide. Without adhesion sites cells remain in tightly packed aggregates (not shown).

Methods

3T3 fibroblasts were cultured in 30 µl *3-D Life* PVA Hydrogel crosslinked with either *3-D Life* PEG-Link or *3-D Life* CD-Link at a crosslinking strength of 2 mmol/l maleimide (Maleimide PVA) and 2 mmol/l SH (PEG-Link or CD-Link) groups. Before crosslinking, Maleimide-PVA was covalently modified with 1 mmol/l *3-D Life* RGD Peptide.

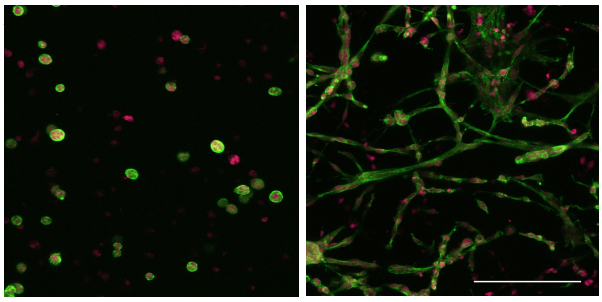

Figure 2: Confocal laser scanning microscopy of 3T3 fibroblasts cultured 14 days in 3-D Life PVA-Hydrogel modified with RGD peptide and crosslinked with either 3-D Life PEG-Link (left) or 3-D Life CD-Link (right). Pictures show collapsed stacks of confocal frames representing a height of 300 µm of the gel. Red: nuclei; green: actin cytoskeleton. Scale bar: 200 µm.

After cell cultivation cells were fixed in 4 % para-formaldehyde in PBS (+ Ca/Mg) for 30 min and washed twice for 10 min in PBS. Cells within the gel were permeabilized in 0,1 % (v/v) Triton® X-100 for 10 min and washed two times with PBS. Cells were incubated with 1,7 µg/ml phalloidin FITC (Sigma) for 1 hr in the dark. Gels were washed with PBS three times 10 min and subsequently incubated 20 min with 1 µmol/l Syto 59 Red (Invitrogen) for nuclei staining. Gels were washed three times 10 min with PBS and stored in PBS at 4°C in the dark. Cells in the gel were visualized by confocal laser scanning microsopy.

Products used

3-D Life Maleimide-PVA, 3 ml, Cat. No. M80-3
3-D Life RGD-Peptide, 3 mg, Cat. No. P10-3
3-D Life PEG-Link, 1 ml, Cat.No. L50-1
3-D Life CD-Link, 1 ml, Cat. No. L60-1
3-D Life User Guide