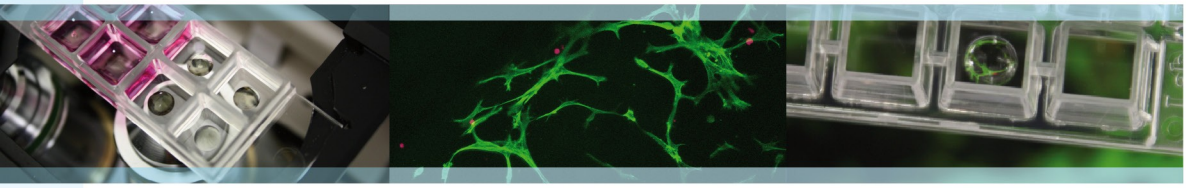

CELLENDES
Cell • Environment • Design

Application Note 1:
Epithelial Cyst Formation in *3-D Life* Hydrogels

RGD Peptide induces cyst formation

Cultivation of renal epithelial cells (MDCK) in *3-D Life* PVA Hydrogels modified with the adhesion peptide RGD induces the formation of a single layer of polarized cells enclosing a lumen (Fig. 1, right). This morphogenesis is comparable to that observed in natural collagen. Without peptide modification cells form a dense aggregate of unpolarized cells (Figure 1, left).

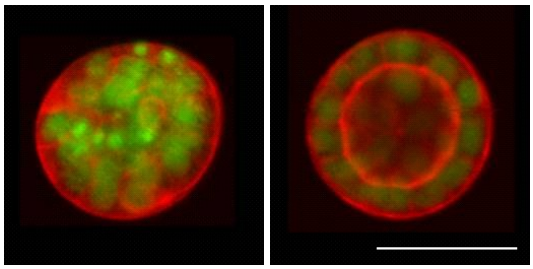

Figure 1: Confocal laser scanning microscopy of MDCK cells cultured 9 days in 3-D Life PVA Hydrogel modified with 5 mmol/l thioglycerol (left) or 5 mmol/l RGD peptide (right) as described in Methods. Red: actin cytoskeleton; green: nuclei. Scale bar: 50 µm.

The extent of cyst formation depends on the RGD peptide concentration

The extent of formation of epithelial cysts depends on the concentration of RGD peptide immobilized in the PVA hydrogel (Fig. 2). Cyst formation starts at 0.5 mmol/l RGD peptide and reaches nearly 100 % at 50 µmol/l. A scrambled version of the RGD peptide (RDG) induces cyst formation in a fraction of cells at 500 µmol/l, but does not reach 100 %, even at 5 mmol/l peptide concentration. This result demonstrates that adhesion in *3-D Life* hydrogels is highly specific, since a control peptide with a scrambled RGD sequence induces a lower percentage of cells to form cysts at much higher peptide concentrations.

Methods

MDCK cells were cultured in 30 µl *3-D Life* PVA Hydrogels crosslinked with PEG-Link at a crosslinking strength of 3 mmol/l (Figure 1) or 4.5 mmol/l (Figure 2) maleimide (Maleimide-PVA) and SH (PEG-Link) groups. Gels contained between 0.05 µmol/l and 5 mmol/l RGD or scrambled RGD peptide, or 5 mmol/l thioglycerol (control.) Gels were prepared as described in the *3-D Life* User Guide. After 9 days of culture, cells in the hydrogels were fixed with 4 % paraformaldehyde in PBS for 30 min and washed two times for 10 min in PBS. Cells were permeabilized with 0,1 % (v/v) Triton® X-100 in PBS for 10 min and washed two times for 10 min in PBS.

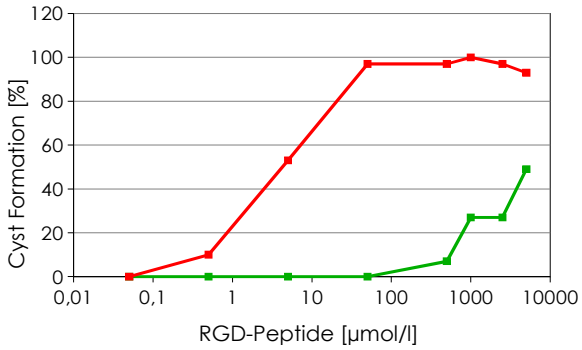

Figure 2: Cyst formation increases with increasing concentration of RGD peptide in PVA hydrogels. Red: RGD peptide; Green: scrambled RGD peptide. n=100 for each peptide concentration.

Gels were incubated with 2.5 µg/ml phalloidin-TRITC (Sigma) in PBS for 1 hr in the dark and subsequently washed three times for 10 min in PBS. Nuclei were stained by incubation of gels with 50 µmol/l Syto-Green® (Invitrogen) for 20 min at room temperature in the dark. Gels were washed three times 10 min with PBS and stored in PBS at 4°C. Cells in the gel were observed by confocal laser scanning microsopy and confocal pictures at different sites of the gel were recorded. For each peptide concentration 100 cell aggregates were analyzed for cyst formation.

Products used

3-D Life Maleimide-PVA, 3 ml, Cat. No. M80-3
3-D Life PEG-Link, 3 ml, Cat. No. L50-3
3-D Life RGD Peptide, 3 mg, Cat. No. P10-3
3-D Life Scrambled RGD Peptide, 3 mg Cat. No. P11-3