CELL SUBSTRATE ELASTICITY IMPACT ON ENDOCYTOSIS

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Introduction

The mechanical microenvironment of cells influences their phenotype and behaviour. *In vitro* studies have established a correlation between substrate viscoelasticity and diverse cell functions including proliferation, motility and differentiation. We have here examined whether substrate elasticity has also an impact on different endocytotic pathways.

Methods

Poly(ethylene glycol) (PEG) hydrogels were prepared via Michael-type addition cross-linking polymerization of multi-arm precursors, end-functionalized with vinyl sulfones (VS) or thiols (SH) [Lutolf, 2003]. Hydrogel elasticity was measured using AFM force spectroscopy and rheology. Fibronectin (Fn) was covalently coupled to the hydrogel surface using sulfo-SANPAH chemistry to promote adhesion of rat embryonic fibroblasts, used as a model cell line. The amount of different fluorescently labelled internalization markers was quantified using flow cytometry. Transfection efficiency of cells cultured on hydrogels was additionally measured by fluorescence microscopy.

Results

The viscoelastic properties of the hydrogels could be tuned by varying precursor molecular weight and concentration; we selected 3 gel compositions for further experiments (Table 1). The amount of coupled Fn on different gels was comparable as measured by immunofluorescence microscopy (Table 1).

Hydrogel	Young's modulus (kPa)	FN intensity
VS(20k)-SH(5k)-5%	5.5 ±0.7	104 ±38
VS(20k)-SH(10k)-10%	33.2 ±5.8	93 ±16
VS(20k)-SH(10k)-20%	65.4 ±8.8	83 ±20

Table 1: Hydrogels used in this study

REF cells attached and proliferated on FN-coated hydrogels. Their spreading and focal adhesion areas increased with increasing stiffness corroborating previous observations on different gels and validating the PEG materials used here.

The amount of labeled transferrin (Tf: *marker of clathrin-mediated endocytosis*), cholera toxin subunit B (CTb: *lipid raft endocytosis*) and dextran 70k (Dex: *non-specific endocytosis marker*) after 1-hour incubation did not significantly differ on cells cultured on the different hydrogels (Figure 1). Transfection efficiency with a commercial agent (Promofectin) resulted in the same fraction of cell transfection independent of substrate elasticity.

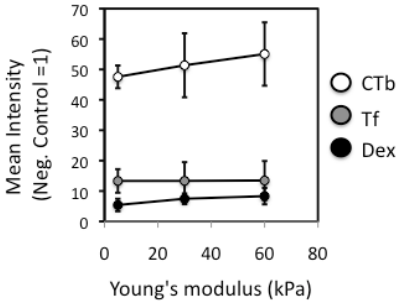

Figure 1: Normalized mean fluorescence intensity per cell as a function of substrate elasticity obtained by flow cytometry measurements. Each data point represents the mean of 3 independent experiments ± SD (for each experiment >5000 cells were analyzed).

Discussion

Our results demonstrate that endocytosis is not dependent on the substrate elastic properties for the REF cell line. Flow cytometry allowed a more robust quantification of uptake compared to microscopy techniques; we believe this is a potential reason for the contradictory results from previous studies that suggested an increase in internalization and transfection on stiffer hydrogels (e.g. [Kong, 2005]). Further reasons could be the differences in adhesion ligand type and density and in cell type used.

In summary our results suggest that endocytosis is a constitutive process not affected by cell substrate elasticity.

References

Lutolf and Hubbell, Biomacromolecules, 4:713-722, 2003.

Kong *et al*, Nat Materials, 4:460-464, 2005.