

## Live-cell monitoring tools for cell-surface interaction investigations

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**INTRODUCTION:** The optimal interaction between cells and implant surfaces is of key importance for the clinical success of implants. Latter interaction can be described as a sequence of time dependent processes. The common biochemical analysis of the biological response taking only mean total culture values and only one or a few time points into account enables only a rough estimate of the latter response. For more detailed description new single cell based tools have to be developed.

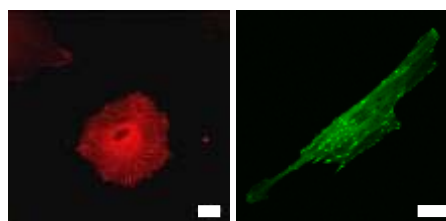
One aspect of cell –surface interaction is the adhesion and spreading behaviour of cells. Cell shape and regulation of biological processes such as proliferation and differentiation are to a large degree connected<sup>1,2,3,4</sup>.

Cell spreading requires a firm contact with the underlying substrate, with focal contacts (FC) being the primary sites of adhesion. They consist of a large number of clustered transmembrane proteins (integrins). FC integrins connect the cell cytoskeleton with the cell substratum. The gradual process of osteogenesis can be followed by different proteins being expressed at various time points, comprising early (e.g. runx2) and late (e.g. osteocalcin) genes.

The aim of our project is to develop new tools to live monitor cell functional states and differentiation of single cells for the investigation of cell – surface interactions.

**METHODS:** We used gene constructs containing the genetic information for the focal adhesion proteins vinculin and vinexin (pEGFP-vinculin, pGFP-vinexin) fused to red or green fluorescence for nucleofection of human bone marrow cells. This method allows fluorescently tagged functional proteins to be visualised and monitored during FC formation and disintegration. Nucleofection allows the delivery of the gene of interest directly into the nucleus and cellular distribution of fluorescence was verified after 1-4 days. In addition cells were transfected with a gene construct reporting for osteogenesis, e.g. osteocalcin.

**RESULTS:** Adult human stromal cells (HBMC) transfected with the fluorescent-labeled vinculin or vinexin showed the expected accumulation of fluorescence signal at focal adhesion sites. The correct localisation of the vinculin was confirmed by staining against endogenous vinculin, suggesting that the tagged protein is correctly synthesized. GFP expression regulated by osteogenesis specific promoters could be detected in differentiated HBMC osteoblastic cells.



*Fig. 1: Transfection of human cells with proteins vinculin (left) and vinexin (right) fused to red and green fluorescent protein. [Scale bar 20 µm] Cells showed the expected accumulation of fluorescence in focal adhesion sites.*

**DISCUSSION & CONCLUSIONS:** Our primary results suggest that transfection of human cells with the present fluorescent-labelled adhesion proteins is efficient and therefore qualified for live cell monitoring.

### REFERENCES:

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