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UNIVERSITÄTSmedizin. MAINZ

Biomaterials, Tissues and Cells in Surgery
Universitätsmedizin Mainz

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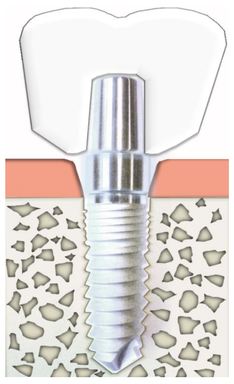
ENHANCEMENT OF OSTEOGENIC CELL RESPONSE ON BIOMIMETIC IMPLANT COATINGS



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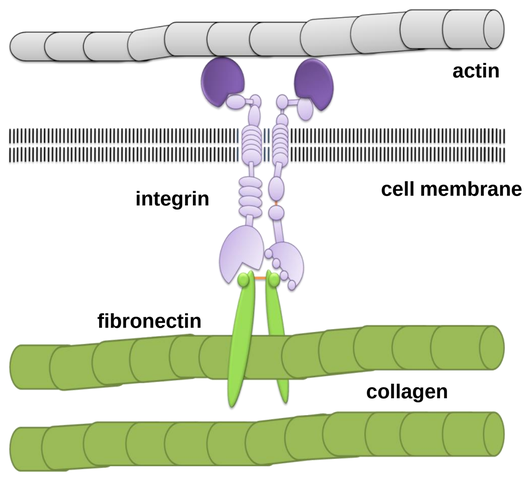
BioNanotechnology

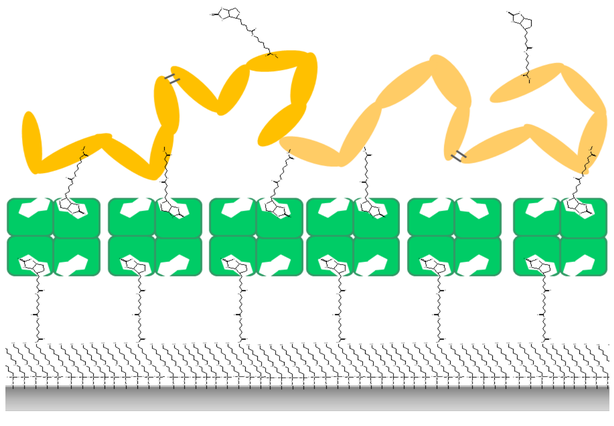
Introduction

Titanium has been used over the last decades as material for bone-anchored implants. However, there are still cases of implant failure. A possible improvement can be the coating of the implant surface with biological coatings using proteins or peptides.

Fibronectin (FN) is an adhesion molecule from the extracellular matrix (ECM). In contact with cellular integrin receptors, FN forms stabile connections between the ECM and the cells. Therefore, FN is an interesting material for biomimetic implant coatings.

Biotinylated FN (bFN) has been immobilized on TiO_2 surfaces in a stabile and flexible way with preservation of biological activity using a Biotin-Streptavidin system.





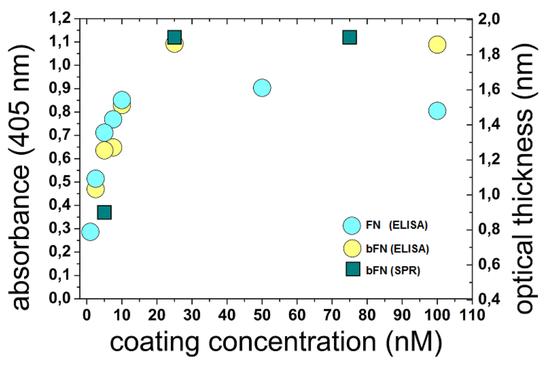
Methods

1. Biomimetic coating of bFN over a Biotin-Streptavidin system *via* **self assembly**
2. Investigation of adsorption conditions by **SPR** (surface plasmon resonance) spectroscopy
3. Investigation of physicochemical properties by **ELISA** (enzyme linked immunosorbent assay)
4. Investigation of cell proliferation and differentiation by **RT-qPCR** (Quantitative Real Time-polymerase chain reaction)
5. **Cell adhesion** tests with HOBs (human osteoblasts) cells

Results

Adsorption behaviour & surface density

Adsorption isotherms of bFN and FN



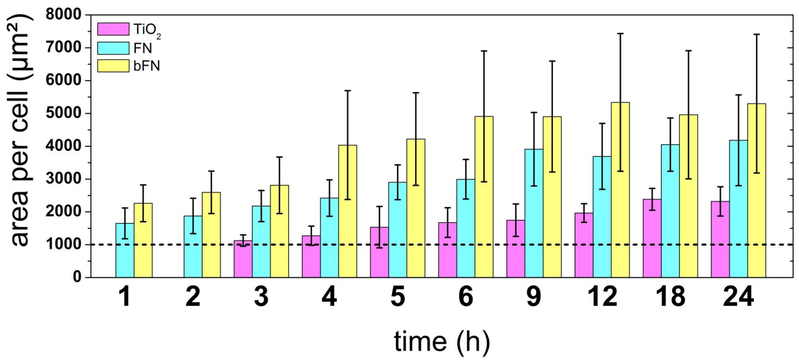
Saturation limit and Surface density

	Saturation Limit	Surface Density
FN	**10 nM**	**0.36 µg/cm²**
bFN	**25 nM**	**0.88 µg/cm²**

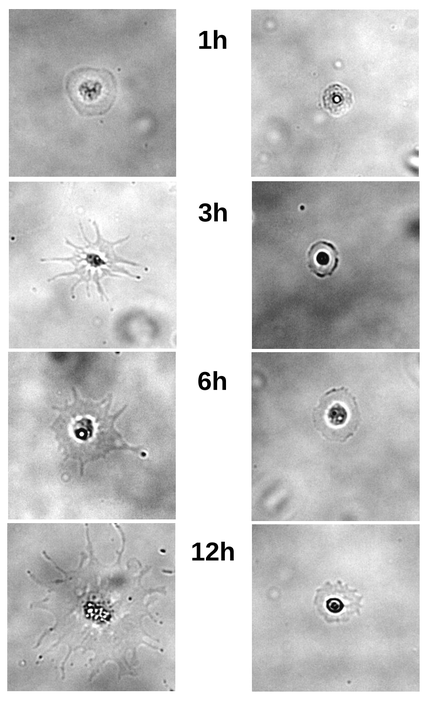
→Saturation limits were detected at coating concentrations of 25 nM for bFN and 10 nM for FN with surface densities at 0.88µg/cm² for bFN and at 0.36µg/cm² for FN.

Cell Adhesion

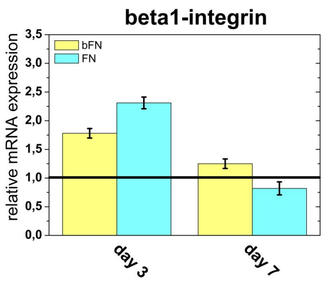
Cell area of HOB on TiO_2, bFN and FN



Initial cell morphology of HOB on bFN (left) and TiO_2 (right)



Cell adhesion of HOB on bFN and FN (normalized to 18S and compared with TiO_2)



→Cell adhesion as a function of cell area was enhanced on bFN and FN coatings.

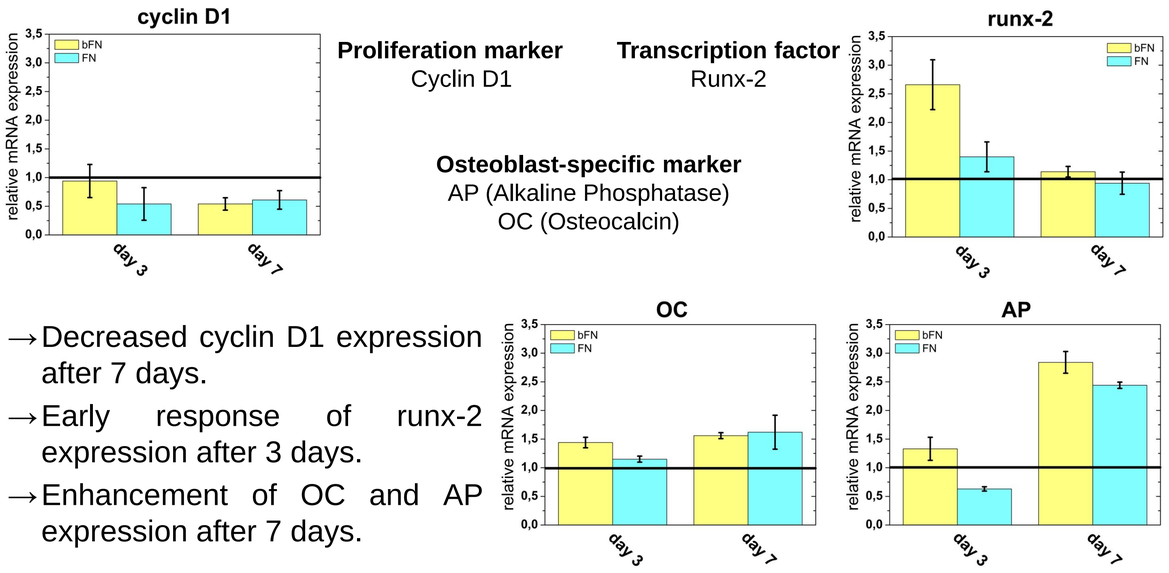
→Initial cell morphology showed faster cell growth and greater cell spreading on bFN compared to TIO_2.

→Expression of β1-integrin was increased on bFN and FN after 3 days and normalized after 7 days.

Cell Proliferation

Cell Differentiation

Cell proliferation and differentiation of HOB on bFN and FN (normalized to 18S and compared with TiO_2)



Proliferation marker
Cyclin D1

Transcription factor
Runx-2

Osteoblast-specific marker
AP (Alkaline Phosphatase)
OC (Osteocalcin)

→Decreased cyclin D1 expression after 7 days.

→Early response of runx-2 expression after 3 days.

→Enhancement of OC and AP expression after 7 days.

Conclusion

- Surface density and saturation limit were two-fold higher for specific immobilized bFN than for unspecific adsorbed FN [1].
- Adsorption isotherms observed with ELISA confirmed previous SPR measurements [2].
- Biomimetic coatings with bFN and FN enhanced osteogenic cell response and triggered early cell differentiation after 3 days.
- Due to this, cell proliferation decreased after 7 days [3].
- Cell adhesion tests indicated a promotion of cell area and β1-integrin expression on bFN and FN.
- Initial cell morphology showed also an enhancement of cell spreading on bFN compared to TiO_2 [3].

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