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Characterization of the adsorption behaviour of biotinylated Fibronectin on a Streptavidin coated surface using ELISA technique

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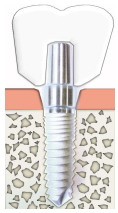
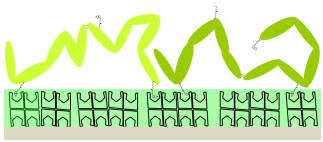


BioNanotechnology

Introduction

The osseointegration of dental implants can be enhanced by appropriate surface modifications. However, there is a risk for periimplantitis due to insufficient attachment of gingival fibroblast cells [1]. The aim of this project is to enhance formation and binding of osteoblasts and fibroblasts to model implant surfaces by biofunctionalisation of the interface.



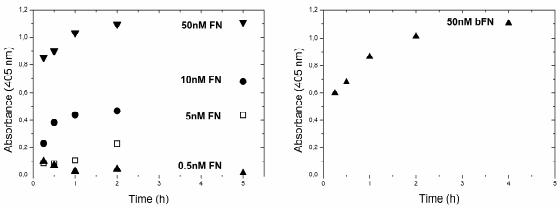
Streptavidin-Biotin system on PS with bound bFN

Approach

Binding of biotinylated Fibronectin (bFN) to a Biotin/Streptavidin layer
Characterisation by Surface Plasmon Resonance (SPR) Spectroscopy
Analysis of the surface coverage using a modified ELISA test with Streptavidin coated Polystyrene (PS)
Compare results with adsorption of Fibronectin (FN) on uncoated PS

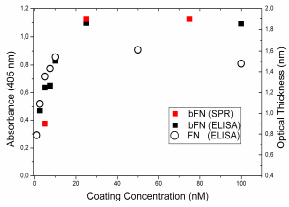
Results

Adsorption behaviour *versus* incubation time



- Rapid increasing of adsorption for both FN and bFN
- Reaching saturation limit after four hours of incubation time latest

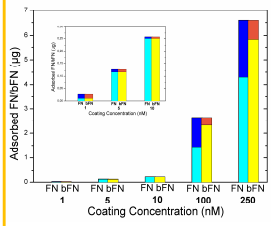
Adsorption behaviour *versus* coating concentration



- For low concentrations FN and bFN behave similar.
- Near the saturation limit, bFN reaches a higher density than FN.
- Both ELISA and SPR yield similar values for the saturation limit

Surface density *versus* coating concentration

Surface density of FN and bFN



Coating Concentration [nM]	Surface Density [µg/cm²]	
	bFN	FN
1	0.01	0.02
5	0.16	0.17
10	0.36	0.36
100	3.35	2.09
250	8.36	6.19

- For lower concentrations, the amount of adsorbed FN and bFN increases linearly with concentration. The obtained surface densities for FN and bFN are equal.
- For lower concentrations all FN/bFN molecules absorb.
- For concentrations above 100 nM, the obtained surface density is significantly higher for bFN.

Conclusion

The time and concentration dependent adsorption behaviour of FN on PS and bFN on Streptavidin are comparable.

Biotinylation has no negative influence on the adsorption behaviour [2]

Data of previous SPR analysis [3] could be confirmed by ELISA. A saturation limit for adsorption of bFN on Streptavidin at 25 nM bFN concentration is found.

Quantification of adsorbed amounts of FN and bFN show a linear correlation with coating concentration. Increase of surface density above mentioned saturation limit suggest that FN and bFN tend to build up multilayer in agreement with Pitt et al. [4].

References

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Acknowledgements

The authors thank Prof. Dr. Loidl-Stahlhofen for helpful comments and discussion and Simon Tröder for excellent technical assistance. This project is supported by a grant (FKZ1775X05) from the FH³ BMBF program through the cooperative project „Bionanofunktionalisierte Oberflächen“.

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