

## IMMOBILIZATION OF OLIGONUCLEOTIDES ONTO SUBSTRATES FOR CELL ADHESION STUDIES

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**INTRODUCTION:** The attachment of biomolecules onto solid supports is very significant for many biotechnological applications such as microchips or biosensors. There are several methods in the literature describing the immobilization of oligonucleotides onto different substrates. The covalent immobilization provides stable, predictable and irreversible attachment of oligonucleotides to a surface. Unfortunately, physical chemistry investigations are still missing.

**METHODS:** We used the method of surface modification by silanizing with APTES using glutaraldehyde as a linker. This method has been reported to be well-suited for immobilization of oligonucleotides. Glutaraldehyde reacts via its aldehyde terminations with the amino groups of APTES, attached on a surface and the amino groups of modified oligonucleotides as well. Silicon wafers were cleaned ultrasonically in chloroform. The samples were activated in a UV/ozone chamber during 15 min. Silanization was done in toluene. The silanized wafers were incubated in a glutaraldehyde solution (pH 4, 24 h, RT).

Subsequence immobilization of the oligonucleotides (aminoC<sub>3</sub>AGAGAGAGAGGGAGAGAGAGAGG G) or PEG onto the modified surfaces (1 h, RT) was performed. To crosslink all non-reacted glutaraldehyde, the samples were immersed in a solution of propylamine in phosphate buffer.

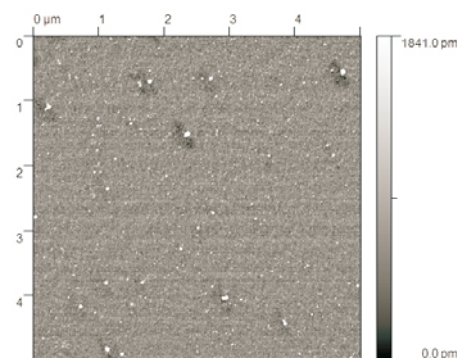
**RESULTS:** Each steps of the preparation were controlled by AFM, FT-IR, contact angle measurement and optimal conditions for these reactions were evaluated. Band position and mode assignments of the characteristic peaks of infrared spectra for the samples at each step of the preparation showed that sequential immobilization of species occurred. AFM images demonstrated the homogeneous coverage.

**DISCUSSION & CONCLUSIONS:** We optimized the method of silanization and glutaraldehyde coupling to get layers in preference covalently linked to the silicon surface. The data obtained showed that the oligonucleotides bind to

glutaraldehyde-modified substrates with preservation of their functional properties. These oligonucleotides can be recognized by cells. The presented experiments are a first step in developing a comprehensive understanding of the role of the immobilized oligonucleotides in the mechanism of cell adhesion.

*Table 1. Average ellipsometry and wettability results for each step of the preparation: silicon wafer before (A), after silanization with APTES (B), after glutaraldehyde binding (C) and after oligonucleotide deposition (D).*

	A	B	C	D
Static contact angle	26.60°	67.15°	55.84°	29.50°
Thickness (theoretical)		0.8 nm	1.4 nm	10.1 nm
Thickness	2.9 nm	1.0 nm	0.7 nm	8.6 nm



*Fig. 1: AFM topographic image of the surface after oligonucleotide immobilization.*

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