Development of a generic biointerface for in flow detection of pathogenic bacteria

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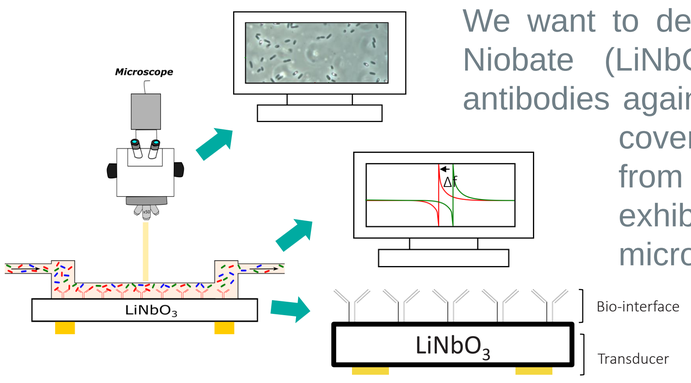
Introduction

The contamination number by microbial agents (bacteria, viruses…) has increased in the food and health industry. It is necessary to detect and quantify these biological elements in complex fluids. It needs to be done in a short time with high selectivity and if possible with a moderate cost. Biosensors seem to meet a number of these criteria, especially with new very efficient piezo electric materials such as AsGa or $LiNbO_3$. None the less, surface chemistry can also help the biodetection efficiency with more and more generic and specific biointerfaces.

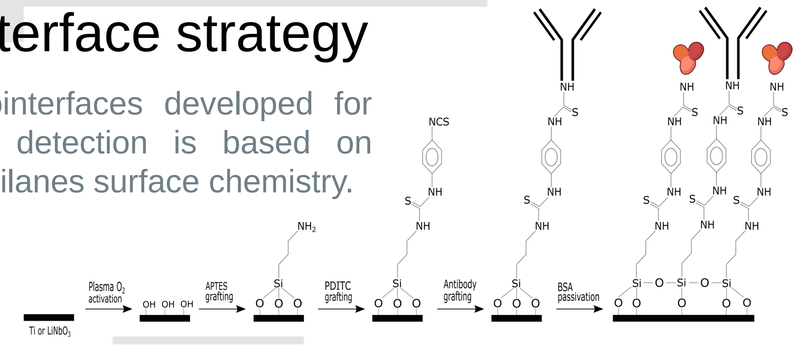
Biosensor design



We want to develop an acoustic biosensor made of Lithium Niobate ($LiNbO_3$). One side will be functionalized with antibodies against the targeted bacteria, the other side will be covered by electrodes to detect the electrical signal from $LiNbO_3$. In addition, the fluidic chamber will exhibit a window in order to perform in situ optical microscopy while recording in real time the transduction signal.

Biointerface strategy

The biointerfaces developed for specific detection is based on organosilanes surface chemistry.

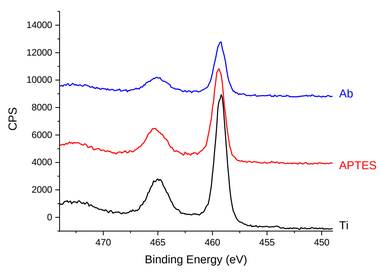


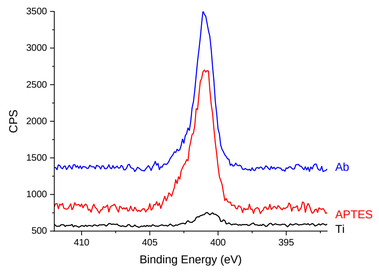
However, some routes need heating (100-150 °C) harmful for some pyroelectric materials such as $LiNbO_3$.
Needs for a Room Temperature strategy → chloroform based route.

Characterisation of biointerfaces

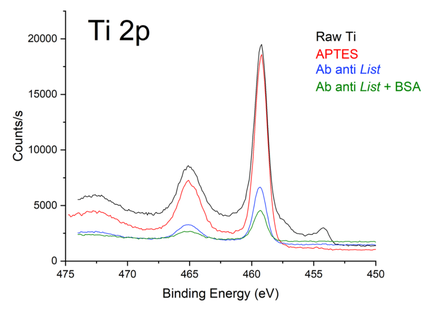
Different surface characterisation techniques are used to check at each step of the process if the grafting was successful: Water contact angle (WCA), IR spectroscopy (FTIR-ATR) and X-ray photoelectron spectroscopy (XPS), all confirming successful grafting of antibodies on the different surfaces. XPS allows some quantitative calculations, namely the quantity of antibodies per area:

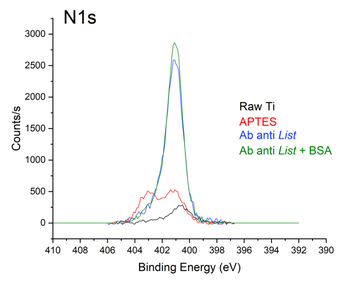
Ab/Titanium toluene route



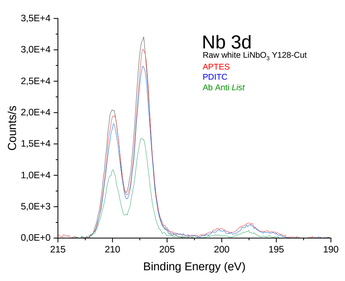


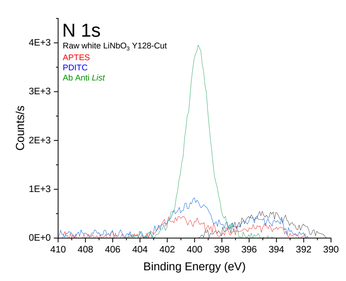
Ab/Titanium chloroform route





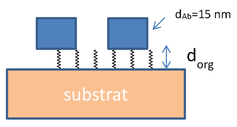
Ab/$LiNbO_3$ chloroform route





Evaluation of Antibodies coverage by XPS

θ : surface covered by Antibodies (Ab)



$$\frac{I_{N1s}}{I_S} = \frac{1320.\frac{\rho_{Ab}}{M_{Ab}}.\sigma_{N1s}.\lambda_{N1s}^{Ab}.\theta.\left[1-exp\left(-\frac{d_{Ab}}{\lambda_S^{Ab}}\right)\right]}{\frac{\rho_S}{M_S}.\sigma_S.\lambda_S^S.\left[\theta.exp\left(-\frac{d_{Ab}}{\lambda_S^{Ab}}\right)+(1-\theta)\right].exp\left(-\frac{d_{org}}{\lambda_S^{org}}\right)}$$

	Ti/Toluene	Ti/Chloroform	$LiNbO_3$/Chloroform
Surface coverage (θ)	85 %	86 %	76 %
Antibodies/µm²	3,8 x 10^3	3,8 x 10^3	3,3 x 10^3

All 3 routes show the same functionalization results
Same quantity of Antibodies/area

Static biodetection of pathogenic bacteria

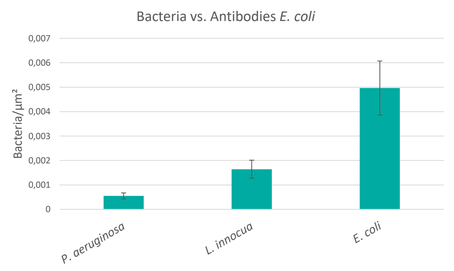
Optical microscopy after staining with cristal violet.
Numbering of bacteria on 30 images, Image J® counting.

Negative control (determined by western blot)

Reactive bacteria (determined by western blot)

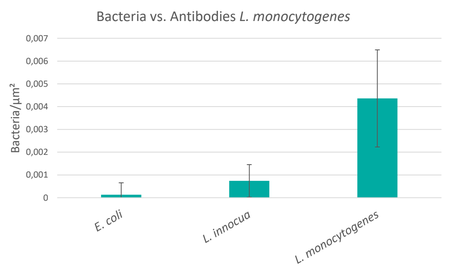
Specific bacteria (determined by western blot)

Titanium toluene route



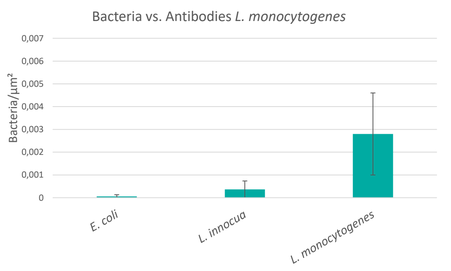
Detection specificity* = 89%

Titanium chloroform route



Detection specificity* = 97%

$LiNbO_3$ chloroform route



Detection specificity* = 98%

All 3 routes show a specificity of detection > 89%. Both chloroform routes show a similar detection efficiency 1,14 x 10^{-7} vs. 0,85 x 10^{-7} bact/Ab for Ti and $LiNbO_3$, respectively.

* specificity = specific bacteria/negative control x 100

Conclusions

We succeeded on the grafting and immobilisation of antibodies on Titanium and $LiNbO_3$ surfaces via a Room Temperature generic biointerface strategy using chloroform as solvent for organosilanes based chemistry.

Static detection of pathogenic bacteria is achieved with up to 97% specificity.

Perspectives

Biodetection experiments to be performed in dynamic to confirm successful and specificity,

Design of fluidic chamber and fabrication of $LiNbO_3$ transducer

Poster C15 . T. Zwingelstein

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