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Master of Science HES-SO in Life Sciences

An innovative bioanalytical method for the screening of antibiotic resistance and antibiotic susceptibility testing

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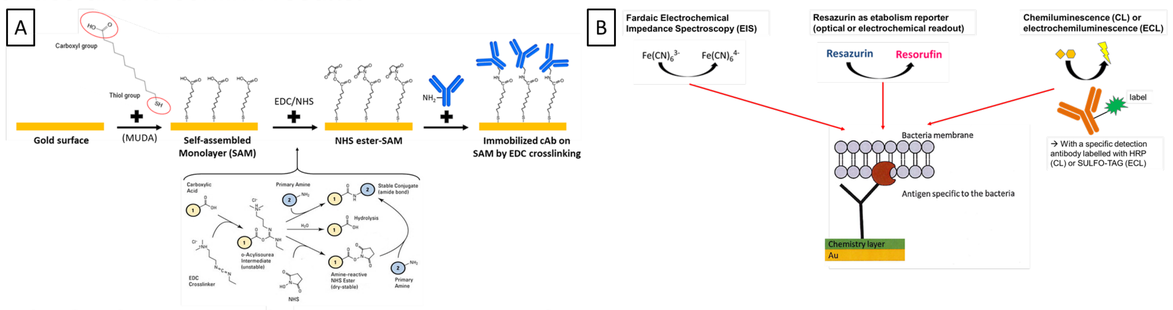
Context and scope of the thesis

Nowadays, bacterial infections remain an actual and serious threat for human health. The actual standard procedure to treat patient bacterial infections is the prescription of antibiotics. But the problem here is that the standard antibiotic susceptibility testing is time consuming (it takes few days to a week). Indeed, the gold standard method that is currently used in hospitals relies on many bacterial culture and sub-culture steps, followed by microorganism phenotyping and standard antibiotic susceptibility tests such as disk diffusion or Luria-Bertani (LB) broth serial dilutions. Broad-spectrum antibiotics are used in the meantime and it induces misuses and overuses of them which leads to development of drug resistant pathogen or the treatment can just simply not be efficient and appropriate at all, leading to mortality.

In this actual context of misuse and overuse of antimicrobial drugs, i.e., antibiotics, to tackle bloodstream and urinary bacterial infection, the development of methods to move from the actual time-consuming gold standard method towards rapid and effective techniques to perform antibiotic susceptibility testing would be a major progress in the current healthcare. The aim of this thesis is therefore to study and improve detection of bacteria based on immunoassay and multiple advanced detection methods such as impedance, chemiluminescence, and electrochemiluminescence with the further purpose to implement it to blood or urinary tract infection in this specific context. The purpose is to find relevant and elegant Point-of-Care approaches to further use it as a basis and allows the further development of an innovative bioanalytical method for the screening of antibiotic resistance and antibiotic susceptibility testing.

Introduction

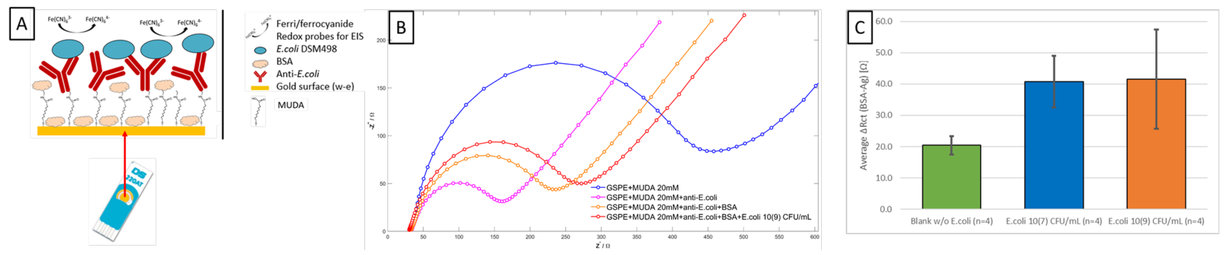
Biosensors shows the advantages of cost effectiveness and fast response when compared to other and standard analytical device. Hence, biosensors show a great interest to be applied as mean for the detection of bacteria in a POC approach. It was indeed decided to assess along this work the detection of bacteria based on immunosensors to capture and detect them. To this purpose, screen-printed gold electrodes from DropSens were used as substrate and transducer to build immunoassays to capture and detect bacteria. Specific antibody was used as biorecognition element and was attached onto the biosensor surface through covalent binding. It was done using carbodiimide chemistry on Self-assembled monolayer (SAM) of MUDA coated onto gold surface. Indeed, thiolated molecule of SAM will form a Au-S bond with gold surface and the antibody is further covalently attached to the SAM molecule through amide bond formation between amine groups of the ligand and carboxylic moieties of the SAM. This is achieved by using carbodiimide chemistry with EDC as crosslinker and NHS to form a stable NHS-ester. The figure bellow on the left describe it into more details. Once the biorecognition element is attached onto the biosensor surface, the antigen, bacteria in this case, can be captured and detected following different detection method such as impedance, with faradaic electrochemical impedance spectroscopy (EIS), (electro-)chemiluminescence (CL or ECL) or also through the use of a metabolism reporter such as resazurin. EIS was used as the main detection method in this work and CL & ECL were also assessed to characterize the biosensor surface and to confirm results.



A) Surface modification strategy of the biosensor to covalently attach the biorecognition element. Done through carbodiimide crosslinking onto MUDA as SAM; B) Overview of the assessed detection methods

Results

Faradaic EIS detection of bacteria was therefore on MUDA-modified SPGE with covalent binding of a specific *anti-E.*coli antibody, ab31499, though carbodiimide crosslinking. A scheme of the assay is shown in the figure bellow. Bacteria suspension of *E.coli* DSM498 was tested at different concentration in PBS; 0, as blank, 10^7 and 10^9 CFU/mL. Faradaic EIS with 5 mM $[Fe(CN)_6]^{-3}$ / $[Fe(CN)_6]^{-4}$ was measured on AutoLab to assess the effect on the impedance of each added layer, including the SAM with 20 mM MUDA, the antibody functionalization (10 µg/mL), the blocking with BSA 5% and the antigen. Data are represented by typical Nyquist plot or as box plot to compare the results of the Δcharge-transfer resistance (Rct) obtained between the previous step, the blocking, and with the antigen incubated. These results are shown in the following figure.

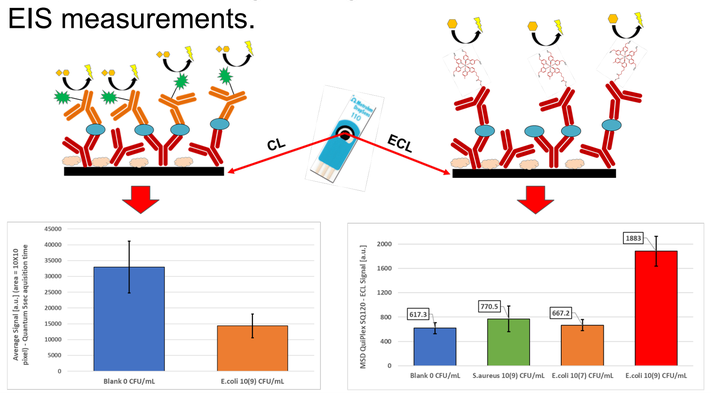


A) Scheme of the EIS detection of E.coli onto SPGEs; B) typical result obtained for the detection of E.coli at 10^9 CFU/mL with Nyquist plot of the different layers: SAM (blue), antibody (purple), blocking (orange) & antigen (red); C) Comparison of the ΔRct obtained with blank at 0 CFU/mL and suspensions at 10^7 & 10^9 CFU/mL in PBS.

Surprisingly, a decrease of the charge-transfer resistance after incubation with the capture antibody can be observed in this result. These high Rct value of the MUDA-modified electrodes can be explained by electrostatic repulsion due to deprotonation of MUDA molecules at the measurement pH, about 7.4. These negative charges of the carboxylates were neutralized after further modification with the carbodiimide. It explains why the charge-transfer resistance was decreasing after the antibody functionalization instead of increasing, as normally expected.

Besides, *E.coli* suspension showed higher ΔRct that the blank at 0 CFU/mL. This suggest that bacteria were captured onto the biosensor. However, these responses are quite low, especially with such high assessed bacteria concentrations. These results are thus not promising yet and it appears that the assay suffers at this point from a clear lack of sensitivity. This means probably that there is not a lot of bacteria that were captured onto the immunosensor and that it would require to determine where comes from this capture issue.

Assays with CL and ECL were therefore performed on similar sensor design to characterize the efficiency of the immunoassays. The results of both are shown in the figure bellow, CL on the leftt-side and ECL on the right-side. The CL results suggest that the used HRP-labelled dAb, ab68450, did not recognized the assessed *E.coli* strain since lower CL response was obtained as if the antigen was not recognized and act as a blocking for NSB. Besides, ECL results suggested that even if the capture antibody, ab31499 (also used as dAb while labelled with SULFO-TAG for ECL) was more specific for the used *E.coli* strain that for a non-specific bacterial specie, *S.aureus*, this antibody did not efficiently capture the target antigen onto the biosensor, probably due to a lack of biorecognition efficiency. These results then highlighted that the used antibodies were not adapted for the target antigen, which also explains the low response obtained with the faradaic EIS measurements.



CL and ECL detection of E.coli onto SPCEs. CL with E.coli suspension at 10^9 versus blank at 0 CF/mL. ECL assay compared blank without bacteria against E.coli conc. at 10^7 and 10^9 CFU/mL, also with a non-specific specie, S.aureus to assess specificity of the antibody.

Conclusion

It appeared along this work that the detection of bacteria was way more challenging than expected. In fact, the detection of E.coli was not successfully achieved, and the results are not that promising yet. Indeed, every performed CL assay failed to detect presence of bacteria while EIS and ECL immunoassays barely showed a specific signal but with high bacterial concentrations. However, the issue along these assays were highlighted to be due to a biorecognition failure of the antibodies for the antigen that was tested. Both used antibodies were not specific and adapted to efficiently recognize to assess the bacterial strain. It is quite surprising since both antibodies are polyclonal commercially available and specific for E.coli. It is also possible that the problem comes from the used strain that may not exhibit surface antigen that are normally targeted by such standard antibodies. Thereby, as perspective to this work It is suggested to select more appropriate antibodies, especially if we want to keep assessing with the same E.coli strain. Besides, it is also suggested to assess different E.coli strain, including an O157:H7 strain since it is often tested in studies. It would therefore be interesting to assess other strain as well as with the strain that was used in this work for comparison, based on the same immunosensors design, which is still believed to be promising, especially with impedance. In addition, a bacterial model based on CRP-coated microparticle to mimic bacteria features could also be assessed to reduce complexity of bacteria as antigen. Eventually, if further detection of bacteria will be achieved with decent bacterial concentration, an ultimate goal would be to assess AST based on such sensors.