Whole Bacterial Cell Imprinting

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Aims of the STSM action:
Molecularly imprinted polymers (MIPs) are stable, inexpensive and easy to produce. These advantages give MIP a great potential as a substitute for current bio-receptors like antibodies. But a lot of problem regarding to the application of MIP as recognition elements still need to be solved. The investigation of the MIP ability for bacterial detection potentially could be a great and promising scientific niche. The idea of the project is to design and develop of MIP as artificial receptors in order to use them for bacterial detection. Escherichia coli DH5α were used as a model bacterial strain in this study. Different electro polymerization techniques were used to achieve different polymer thickness. Related to the aim of the joint project three functional monomers, Scopoletin, Pyrrole and ortho-phenylenediamine (o-PD), were used for extending of the diversity of the polymers. In our pervious experiments the scanning electron microscope images proved that we had succeed to capture the whole bacterial cells by using electro polymerization of pyrrole and o-PD in presence of the bacterial cells. After the first step of the imprinting process, capturing the target, we needed to find a proper treatment for target removing. So, before any further experiments we investigated different methods for removing bacterial cells from the polymer network. In this purpose, different enzymatic, chemical and electrochemical treatments were examined.

Results:
o-PD is well suited to molecular imprinting, easily electropolymerized on various substrate materials and forms films with good chemical and mechanical stability offering hydrophilic, hydrophobic and basic recognition sites via electrostatic interactions. A thin film (<20 nm) of o-PD was electrochemically deposited on the Gold Wire Electrode (GWE) in presence of bacterial cells by cyclic voltammetry electropolymerization method. Samples were treated by NaOH, H₂SO₄, SDS, Triton X, KOH, KCl and NaCl in various concentration and time. The results shows that the harsh conditions like NaOH and H₂SO₄ in high concentration or long time could damage the polymer network that affected the control test, Non Imprinted Polymer (NIP), while the moderate conditions like KCl, NaCl or low time and concentrations of acidic and alkaline treatments have no effect on polymer and templates. As the best of our results, it’s concluded that bacterial cells should not be entrapped in the polymer body just by simple electrostatics interactions. Existence various groups of proteins and glycoproteins with many snappy molecules at the bacterial cell surface, gives this hypothesis that covalent binds might be happened between polymer and bacterial cells during electropolymerization. As a proper method to overcome the problem, enzymatic treatment was tested to cleavage the bonds between polymer and bacterial cells. Lysozyme and Trypsin were used as the most common enzymatic treatment for generally removing cells from surface. The results shows that these two tested enzymes were not able to completely remove cells from the MIP. Also, during the experiments we found that the enzymes non-specifically adhere to the polymer surface. So, a combination removing process strategy was taken include enzymatic treatments to cleavage bacterial connection with the polymer and in subsequent, Glycine-HCl treatment to remove sticking enzymes to the surface and finally a
chemical treatment for extraction bacterial cells from polymer network. Enzyme treated MIP samples were subjected to treatment with different chemical conditions include NaOH, H₂SO₄, KOH, KCl, NaCl, SDS and Triton X in various concentration and time. Although electrochemical measurements showed some cavity in the polymer network which might be related to the removing of the bacterial template, the magnitude amount of result was not so high to be well distinguished with the background response and also it was not well repeatable. The problem of the magnitude of the response should be related to the low concentration of captured cells to the surface which needs to be optimized by further experiments and reproducibility also might be related to the few number of cavity on the polymer network which decrease the sensitivity of the experiment.

Subsequently, the other functional monomers were used for fabrication of MIP to investigate the difference capability of varying materials for the aim of the project. Polypyrrole is a conductive polymer that is easily synthesized electrochemically by doping anionic bacteria. The physical properties of PPy like roughness, thickness and hydrophobicity of this polymer is tunable which make this polymer as a good material for fabrication of MIP. As previously mentioned here, the bacterial cells had been already captured in PPy network by using cyclic voltammetry electropolymerization technique. Then, different chemical treatments, which used for o-PD, were used here to extract bacterial cells from PPy network synthesised on GWE. No success to remove template by using chemical treatment intensified the hypothesis of the existence of covalent bond between bacterial cell surface and polymer network. Electrochemical treatments, PPy overoxidation, were used in combination of enzymatic and chemical treatments as a new strategy to expel bacterial cells from the polymer network. Overoxidation is irreversible electrochemical oxidative degradation of polypyrrole under an anodic applied potential. Although the intensity of electrochemical response of modified MIP after a three step treatment by enzyme, overoxidation and Glycine-HCl was not so drastically promising, SEM images have already proved that some bacterial cell captured in the polymer network were partially removed. The main problem here related to the week results response might be related to the low concentration of captured bacterial cells to the polymer surface which needs to be optimized in our future work. Also, it seems one more enzymatic treatment after overoxidation could remove remaining parts of the cell wall to the polymer network after overoxidation treatment.

The third monomer which was investigated in this project for fabrication of MIP was scopoletin. MIP electrodeposited by cyclic voltammetry in presence of bacterial cells and all above treatments was used to remove cells from polymer network. Analysis of the results showed that there was no difference between functional monomers in this step of the project.

To shed light on this matter, all these three functional monomers seems to react with bacterial cell wall during electropolymerization and make covalent bind cause removing step to be a great challenge even if using all enzymatic, electrochemical and chemical treatment in combination. In further experiments we focus on broad types of enzymatic treatment and subsequent chemical or electrochemical treatments before and after overoxidation process. We hope after optimizing the removing step, make the process in combination by combining sugar-terminated SAM for bacterial detection.

**Future collaboration:**
Strengthening of the collaboration between Biosensors and Bioelectronics Centre at Linköping University and the department of Analytical Biochemistry at University of Potsdam was one of the aims of the visit. We have discussed about the possibility of future collaboration in the development of whole bacterial cell imprinting.