Popular science abstract

Recently there has been an increased demand for selective identifying microorganisms in many areas of life. In various disciplines such as medicine, water treatment, food safety and security, the detection and identification of harmful bacterial strains found in soil, marine and estuary waters is critical. Infections, particularly those caused by life-threatening pathogenic bacterial strains, have become a major cause of death and increased morbidity among hospitalized patients. Despite several attempts to create antimicrobial treatments, these illnesses continue to pose a significant problem due to the emergence of multidrug-resistant pathogens. To quantify pathogenic and non-pathogenic bacteria, several analytical methods based on colorimetric analysis, polymerase chain reaction (PCR), enzyme-linked immunological assay (ELISA), and mass spectrometry techniques have previously been established. These procedures have some drawbacks, such as being time-consuming, tedious, and expensive. As an example, the traditional antibody-antigen host systems used to identify pathogenic bacteria lack specificity and stability towards the cell walls, that correspond to these pathogens. Therefore, the goal is to design a fast, effective and stable sensor for the determination of specific microorganisms in a given sample.

Molecular imprinting materials have been proposed as viable alternatives to natural recognition systems for improving microbe detection with increased selectivity. Molecularly imprinted polymers (MIP) synthesis is relatively simple and cheap. The chemical properties of MIPs are easily controlled by the synthesis technique and type of compounds used for the reaction. The basic condition for the MIP to work is the creation of cavities in its structure, featuring locations responsible for molecular selective recognition. In most cases, this is done using cross-linking compounds in sufficient proportions to assure MIP stability in traditional bulk imprinting. In bulk imprinting, the template (a microorganism or its part in the case of this project) is combined with a monomer, crosslinker, initiator, and solvent in a pre-polymerization mixture. During this process homogeneously dispersed cavities in the whole polymer matrix are formed (after polymerization and template removal). MIPs allow for the enhancement of detection efficiency and selectivity (molecular cavities correspond to the shape and interactions of the template applied, so they are unique for every bacteria species). Therefore, microorganisms with distinct structures and conformations can be distinguished in biosensing devices with sterically and functionally complementary locations utilizing molecularly imprinted materials. Electrochemical biosensors, particularly amperometric and potentiometric biosensors, are well-documented in the literature. These devices are capable of detecting electrochemical changes that occur as a result of chemical interactions of molecules of the analyte and the sensing surface of the detecting electrode.

The project involves the synthesis of a range of carbon-polymer materials in which molecular imprints can be formed. The hybrid materials produced will be based on graphene oxide (the carbon part) and polyethyleneimine (the polymer part). Graphene oxide, as an oxidized form of graphene, has many functional groups on its surface, which influence its reactivity and easiness of functionalization reaction. Functional groups, that are present on the surface of graphene oxide, concerning molecular imprinting technique, will form additional bonds with the template molecules. Graphene oxide can also be electrically conductive (after sufficiently effective reduction and regeneration of the graphene structure) and, most importantly, has a large specific surface area. On the other hand, polyethyleneimine is a very well-studied polymer that has been successfully used to produce molecular imprint materials. This project will result in the synthesis of materials that will contain different functional groups (and different quantities of them) due to the o previous functionalization of the polyethyleneimine. The influence of functional groups in the polymer and in the overall carbon-polymer composite will be investigated. Despite indications from the literature that have successfully described the generation of molecular imprints using whole cells, the project will apply an epitopic approach – only the outermost bacterial cell wall parts will be used as templates. Difficulties with whole-cell imprints include the following: their enormous size, which causes certain difficulty when detaching attached cells from cavities. Furthermore, imprinted cavities have a substantial impact on microbe detection because cell transport into the matrix is slow, and the resulting diffusion issue lengthens the system's reaction time. Four species of bacteria were selected (2 Gram-positive and 2 Gram-negative) for the project. Gram-negative bacteria - *Escherichia coli and Salmonella enterica* and Gram-positive bacteria - *Staphylococcus aureus, Bacillus subtilis.* Lipopolysaccharides and peptidoglycans that will be used as templates are parts of mentioned bacteria cell walls. Once the molecularly imprinted materials have been synthesized, they will be characterized by cyclic voltammetry and electrochemical impedance spectroscopy. By combining these methods with HPLC it will be possible to determine the concentration-intensity correlation. The quality of the electrochemical biosensors will also be investigated in environmental tests, where measurements will be taken in solutions containing living bacteria. Linearity range, detection and quantification limit, selectivity and reproducibility will be determined to characterize performance of obtained molecular imprinted materials in terms of their application as biosensors.