MAGNETIC MOLECULARLY IMPRINTED POLYMERS USED FOR STAPHYLOCOCCUS AUREUS ISOLATION AND DETECTION

Jaroslava BEZDEKOVA, Jitka HUTAROVA, Marketa VACULOVICOVA

Department of Chemistry and Biochemistry, Mendel University in Brno, Brno, Czech Republic, EU,
jzelnickova@seznam.cz

Abstract

A sensitive detection of microorganisms plays an important role in prevention and identification of infectious and deadly diseases. However, conventional methods of bacterial detection are time-consuming, laborious or expensive. A promising way to overcome these problems is offered by the strategy of molecularly imprinted polymers (MIPs), which uses molecular templates to create selective binding sites in cross-linked polymer layers. By this approach, it is possible to prepare materials tailored for recognition of target analyte taking to account the detection method available. In this work, the novel sensitive method for *Staphylococcus aureus* isolation and detection was investigated. This method utilized the non-covalent molecular imprinting technique employing dopamine as a functional monomer. A layer of molecularly imprinted polymer was created on a surface of magnetic particles (MPs). MPs were chosen due to the ability to pre-concentrate bacteria from large sample volumes and thereby allow for the extremely sensitive detection.

Keywords: Molecularly imprinted polymers, bacteria, dopamine, fluorescence

1. INTRODUCTION

Bacteria belong to a large group of unicellular ubiquitous microorganisms. Due to of their ubiquity, size, and fast growth rates, bacteria rank to the most commonly encountered biological contaminants. Their presence must be monitored and controlled because they can cause serious public health problems [1]. Nowadays, the most common tools for bacterial detection are culture and colony counting methods, polymerase chain reaction, and immunology-based methods as enzyme-linked immunosorbent assay (ELISA) [2]. Unfortunately, these conventional microorganism identification methods have several disadvantages. Counting methods are time-consuming and methods as ELISA use antibodies that are expensive and have low stability [3]. Therefore, there is an effort to develop some alternative method of bacterial detection.

This work is concentrated to detection of bacteria by molecularly imprinted polymers (MIPs). Technique of molecular imprinting uses a template (in this case bacteria) to create selective binding sites in cross-linked polymer [4]. MIPs have a range of advantages, in comparison to other bio-macromolecules as antibody or enzyme, which include high chemical and physical stability, low production costs and possibility of preparation the complementary binding sites for variety types of molecules/particles [5]. Due to of these features, MIPs are ideal choice for a large number of biochemical applications.

For preparation of MIPs in this work, a non-covalent imprinting approach was used. Dopamine (DA) was chosen as a functional monomer because it undergoes an oxidative polymerization under alkaline conditions [6]. Other advantages of using DA are the fact that it is eco-friendly and contains a lot of functional groups that enable the interaction with imprinted bacteria [7]. Layer of molecularly imprinted polymer was prepared on the surface of magnetic particles (MPs). MPs were used because they can be employed for isolation of the analyte from large sample volumes due to high magnetic sensitivity [8]. Gram-positive facultative anaerobic bacterium *Staphylococcus aureus* (S. *aureus*) was chosen as a template. These bacteria cause range of serious infections and therefore, they are a critical threat to public health [9]. Scheme of preparation of S. *aureus* selective MPs is shown in **Figure 1**.

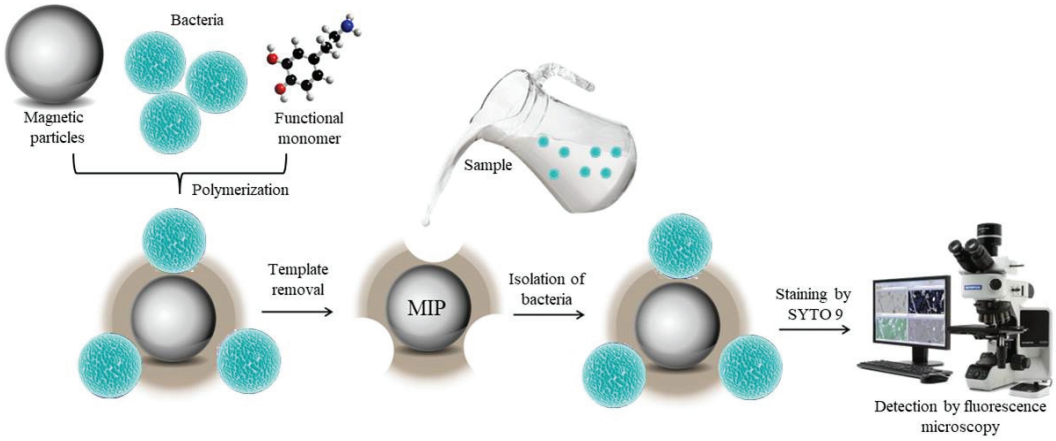

Figure 1 Scheme of preparation of *S. aureus* selective MIPs. The templates (bacteria) were mixed with magnetic particles and functional monomer (dopamine). After polymerization, the templates were removed. Prepared MIPs were used for isolation of analytes (bacteria) from sample. Bound bacteria were stained by fluorescent dye (SYTO 9) and determined by fluorescence microscopy/capillary electrophoresis with laser-inducted fluorescence detection

2. MATERIALS AND METHODS

2.1. Materials

Dopamine hydrochloride, Trizma base, sodium dodecyl sulfate and acetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity. Dynabeads MyOne Silane, tetraborate decahydrate and SYTO 9 were obtained from Thermo Fisher Scientific (Waltham, MA, USA).

2.2. Preparation of bacteria

The bacterial strains of *S. aureus* NCTC 8511 and *Enterococcus faecalis* ATCC 11700 (Czech Collection of Microorganisms, Brno, Czech Republic) were cultivated in Muller-Hinton broth (MHB; Oxoid, Hampshire, UK) overnight at 37 °C and 150 rpm. The concentrations of bacterial solutions were determined by optical density at 600 nm and using the MHB the cultures were adjusted to a concentration of 1×10^6 CFU·ml^{-1}. The cells were centrifuged at 8000 rpm for 10 min at ambient temperature and the supernatant was discarded. The cells were re-suspended in the same volume of 20 mM TRIS pH 8.5.

2.3. Preparation of magnetic molecularly imprinted polymers (MPs-MIPs)

The MPs-MIPs were prepared according the method reported by Yang and co-workers with slight modification [10]. Briefly, 50 µl of Fe_3O_4 nanoparticles (40 mg/ml) were washed three times by 200 µl of 20 mM TRIS (pH 8.5). Subsequently, 600 µl of *S. aureus* (1×10^6 CFU/ml) suspended in 20 mM TRIS (pH 8.5) was added to the washed MPs. To prepare non-imprinted polymers (NIPs) that are used as control, 600 µl of 20 mM TRIS (pH 8.5) was added to the washed MPs. The mixtures were mechanically stirred for 2 hours until the MPs were well suspended. Then, 100 µl of dopamine (17.5 mg/ml) suspended in 20 mM TRIS (pH 8.5) was added and the reaction continued over night at laboratory temperature. Next, the product was collected with an external magnetic field and the template was washed out three times by 200 µl of solution containing mixture of 5 % acetic acid and 1 % SDS and once by 200 µl of MilliQ water.

2.4. Binding ability of the imprinted materials

200 µl of S. *aureus* (1×10^6-1×10^2 CFU·ml^{-1}) suspended in 20 mM TRIS (pH 8.5) was added to the MPs-MIPs and mixture was shook for 1 hour. Then, the unbound bacteria were removed and MPs-MIPs were

washed by 200 µl of MilliQ water. 18 µl of sample (MP-MIPs with bound analyte or MPs-NIPs) was mixed with 2 µl of 5 mM SYTO 9 (dissolved in DMSO) and the mixture reacted for 10 minutes. Next, the mixture was analyzed by capillary electrophoresis with laser-induced fluorescence detection (ex. 488 nm, em. 530 nm) or by fluorescence microscopy (ex. 545 nm, em. 610 nm, dichroic mirror 600 nm).

2.5. Detection of binding analytes

Capillary electrophoresis

Samples were analyzed by CE (Agilent technologies, Waldbronn, Germany) with laser-induced fluorescence detector (ZetaLIF, Picometrics, Toulouse, France) with solid-state laser (λ_{em} = 488 nm) as an excitation source. Fused silica capillary with internal diameter of 75 µm and with the total length of 64.5 cm and effective length of 56 cm was used. The sample was introduced hydrodynamically by 50 mbar for 5 s and separation voltage of 25 kV was applied, 20 mM borate buffer pH 9 was used as an electrolyte. Prior to the analysis, the capillary was washed for 60 s by the separation electrolyte.

Fluorescent microscopy

An Olympus IX71 inverted fluorescence microscope was used for imaging of MIPs/NIPs. The used objective was LUCPLFLN 20 X PH. Total magnification was 200×. Detector of emitted light was Hamamatsu CCD ORCA-HR (C4742-95-12HR) with pixels of 1600 × 1200 was used and exposure time was 4 s. Applied filter was TX Red (ex. 545 nm, em. 610 nm, dichroic mirror 600 nm).

3. RESULTS AND DISCUSSION

3.1. Binding properties of the imprinted materials

To assess the binding properties of created MIPs, the binding experiments were carried out. The binding experiments were accomplished with various initial concentrations of *S. aureus*, ranging from 1×10^6 to 1×10^2 CFU·ml^{-1} and the binding capacities of MIPs against NIPs were compared. Acquired data is shown in **Figure 2**. It was found that the adsorption of bacteria on the surface of MIPs rapidly increased with growing bacterial concentration. On the other hand, the use of higher concentration caused increased binding of analyte on surface of NIPs. NIPs are prepared under same conditions as MIPs. However, NIPs are prepared without presence of the template. NIPs were used as an indicator of non-specific binding. The functional groups in NIPs are randomly organized on the surface and can interact with analyte causing a non-specific interaction. Non-specific interactions are weaker than specific interaction caused by selective binding sites in MIPs created by imprinting of the template molecule. Therefore, NIPs are able to bind lower amount of analyte.

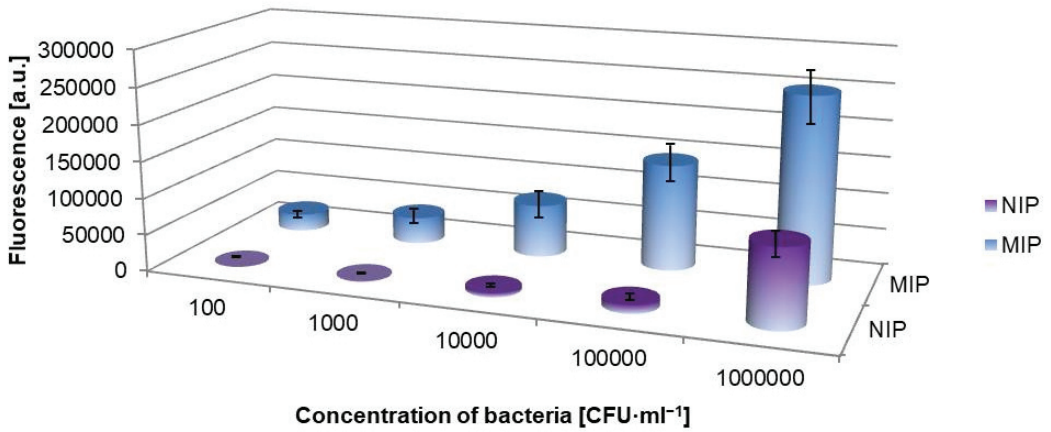

Figure 2 Adsorption isotherm of prepared magnetic MIPs and NIPs selective for *S. aureus*

The binding properties of prepared MPs-MIPs/NIPs were evaluated by two different methods. First of them was fluorescence microscopy. The images of MIPs/NIPs are shown in **Figure 3 A, B**. These images were subsequently evaluated using the BRUKER Molecular Imaging software. From the quantification of the fluorescence images follows that bacterial capture on NIP surface is only 8 % of the capture on imprinted MIP surface.

The second method used was the capillary electrophoresis with laser-induced fluorescence (CE-LIF) (**Figure 3 C**). The MP-MIPs with fluorescently labelled bacteria were directly injected into the capillary and the signal of MIPs as well as NIPs was evaluated based on the electrophoretic peak height. As it was found, the detection using CE-LIF is more sensitive in comparison with fluorescence microscopy.

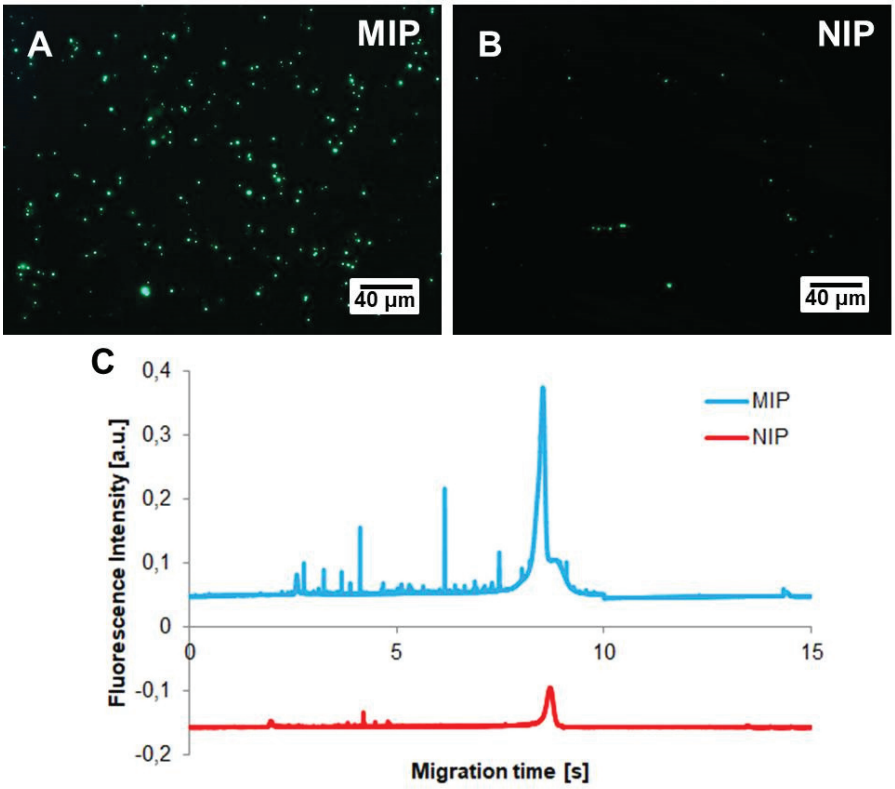

Figure 3 Micrographs of MPs-MIPs (A) and MPs-NIPs (B) with bacteria S. *aureus* (1×10^6 CFU·ml^{-1}) stained by fluorescent dye SYTO 9; Electropherograms of MPs-MIPs/NIPs (C)

4. CONCLUSION

In this work, a new alternative method of bacterial detection based on molecular imprinting was developed. The MIPs selective for gram positive bacteria *S. aureus* were prepared on the surface of MPs. Technique of non-covalent molecular imprinting was used in preparation process. Dopamine was used as a functional monomer. Experiment focused on adsorption efficiency of created polymers was carried out. The prepared imprinted polymers exhibited great binding properties under the optimal conditions. Detection of isolated bacteria was performed by fluorescence microscopy and capillary electrophoresis with laser-induced fluorescence detection. These two methods were compared. It was observed that CE-LIF was more sensitive than fluorescence microscopy. By CE-LIF, we were able to detect very low bacterial concentration (1×10^2 CFU·ml^{-1}). Moreover, developed method is very simple, specific, low-cost and eco-friendly. The presented technique enables fast and easy bacterial detection and could find potential in food industry. Therefore, future work will be focused on isolation of bacteria from real sample (e.g. milk).

ACKNOWLEDGEMENTS

This work has been supported by the Czech Science Foundation (project No. 16-23647Y) and by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601).

REFERENCES

[1] MUMTAZ S., WANG S., HUSSAIN S.Z., ABDULLAH M., HUMA Z., IQBAL Z., CRERAN B., ROTELLO V.M., and HUSSAIN I., Dopamine coated Fe3O4 nanoparticles as enzyme mimics for the sensitive detection of bacteria. *Chemical Communications*. 2017. vol. 53, no. 91, pp. 12306-12308.

[2] LAZCKA O., DEL CAMPO F.J., and MUNOZ F.X., Pathogen detection: A perspective of traditional methods and biosensors. Biosensors & Bioelectronics. 2007. vol. 22, no. 7, pp. 1205-1217.

[3] AHMED A., RUSHWORTH J.V., HIRST N.A., and MILLNER P.A., Biosensors for Whole-Cell Bacterial Detection. *Clinical Microbiology Reviews*. 2014. vol. 27, no. 3, pp. 631-646.

[4] CHEN L.X., WANG X.Y., LU W.H., WU X.Q., and LI J.H., Molecular imprinting: perspectives and applications. *Chemical Society Reviews*. 2016. vol. 45, no. 8, pp. 2137-2211.

[5] VASAPOLLO G., DEL SOLE R., MERGOLA L., LAZZOI M.R., SCARDINO A., SCORRANO S., and MELE G., Molecularly Imprinted Polymers: Present and Future Prospective. *International Journal of Molecular Sciences*. 2011. vol. 12, no. 9, pp. 5908-5945.

[6] JIANG J.H., ZHU L.P., ZHU L.J., ZHU B.K., and XU Y.Y., Surface Characteristics of a Self-Polymerized Dopamine Coating Deposited on Hydrophobic Polymer Films. *Langmuir*. 2011. vol. 27, no. 23, pp. 14180-14187.

[7] IQBAL Z., LAI E.P.C., and AVIS T.J., Development of polymer-modified magnetic nanoparticles and quantum dots for Escherichia coli binding test. *Microchimica Acta*. 2012. vol. 176, no. 1-2, pp. 193-200.

[8] PAN J.M., XU L.C., DAI J.D., LI X.X., HANG H., HUO P.W., LI C.X., and YAN Y.S., Magnetic molecularly imprinted polymers based on attapulgite/Fe3O4 particles for the selective recognition of 2,4-dichlorophenol. *Chemical Engineering Journal*. 2011. vol. 174, no. 1, pp. 68-75.

[9] BOLE A.L. and MANESIOTIS P., Advanced Materials for the Recognition and Capture of Whole Cells and Microorganisms. *Advanced Materials*. 2016. vol. 28, no. 27, pp. 5349-5366.

[10] ZHOU W.H., LU C.H., GUO X.C., CHEN F.R., YANG H.H., and WANG X.R., Mussel-inspired molecularly imprinted polymer coating superparamagnetic nanoparticles for protein recognition. *Journal of Materials Chemistry*. 2010. vol. 20, no. 5, pp. 880-883.