Isolation and detection of bacteria using magnetic molecularly imprinted polymers

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Abstract: A range of biological macromolecules as antibodies, enzymes or receptors function on principles based on selective recognition. These biological macromolecules are very often used in many technical applications due to their well-defined selectivity features. Unfortunately using of biomolecules is limited by their high production costs and low stability. Therefore, an idea of creation of synthetic materials with tailor-made molecular selectivity was presented. These materials are called molecularly imprinted polymers (MIPs). MIPs use molecular imprinting of chosen molecule to creating selective binding sites in cross-linked polymer. Technique of molecular imprinting has become one of the most efficient methods that are used for preparation of selective recognition materials. MIPs are stable, robust and have low production costs. In this work, the novel sensitive method for *Staphylococcus aureus* isolation and detection based on molecular imprinting was investigated. Molecularly imprinted layer was created on a surface of magnetic particles (MPs) due to ability of MPs pre-concentrate bacteria from large sample volumes. Fluorescence microscopy was used for detection of isolated bacteria.

Key Words: molecularly imprinted polymers, magnetic particles, fluorescence, bacteria, dopamine

INTRODUCTION

Molecular imprinting is a technique which enables to create the tailor-made binding sites specific to the template (imprinted target molecule) in shape, size and functional groups in cross-linked polymer (Chen et al. 2016). MIPs have a range of advantages which includes: high chemical and physical stability, possibility of preparation the complementary binding sites for variety types of molecules (Li et al. 2012), production in large quantities and possibility of re-use (Mattiasson and Ye 2015). These features make MIPs ideal for a large number of biochemical applications.

In this work, molecular imprinting technology was used for isolation and subsequent detection of bacteria *Staphylococcus aureus* (SA). A non-covalent imprinting approach is the most commonly used due to the simplicity of preparation (Mattiasson and Ye 2015). Dopamine (DA) can be used as an effective functional monomer for creation of the polymeric layer because it undergoes an oxidative polymerization under alkaline conditions (Jiang et al. 2011). During the polymerization, the reaction occurs to create polydopamine (PDA) layer. The use of DA is beneficial due the fact that it is eco-friendly and contains a lot of functional groups (such as phenyl, amino, and hydroxyl groups) that enable reaction with imprinted molecule (Zhao et al. 2018).

Layer of molecularly imprinted polymers can be prepared on a surface of different types, material, and geometrical arrangements such as silica beads, carbon nanotubes, quantum dots and/or magnetic particles (MPs) (Zhou et al. 2010). MPs can be easily collected by an external magnetic field without necessity of centrifugation or filtration due to high magnetic sensitivity. Therefore they are enable isolate analyte from large sample volumes (Pan et al. 2011).

This work is concentrated on isolation and detection of bacteria. This topic was chosen because control and identification of the bacterial contamination is very important in many industrial branches such as food industry, environment industry or clinical diagnostic. However, current methods are time-consuming, laborious and/or expensive (Lazcka et al. 2007).
MATERIALS AND METHODS

Materials

Dopamine hydrochloride, Trizma base, Sodium dodecyl sulphate and Acetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity. Dynabeads™ MyOne™ Silane and SYTO 9 were obtained from Thermo Fisher Scientific (Waltham, MA, USA).

Preparation of bacteria

The bacterial strains of *Staphylococcus 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 \( \sim 1 \times 10^6 \) CFU/ml. 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(hydroxymethyl)aminomethane (TRIS) of pH 8.5.

Preparation of magnetic MIPs/NIPs

The molecularly imprinted polymers prepared on the surface of magnetic particles (MPs-MIPs) were prepared according the method reported by Yang and his co-workers with slight modification (Zhou et al. 2010). Briefly, 50 µl of Fe3O4 nanoparticles (40 mg/ml) were three times washed by 200 µl of 20 mM TRIS (pH 8.5). Subsequently 600 µl of *Staphylococcus aureus* (1 \( \times 10^6 \) CFU/ml) suspended in 20 mM TRIS (pH 8.5) was added to the washed MPs. For prepare of 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. Than 100 µl of dopamine (17.5 mg/ml) suspended in 20 mM TRIS (pH 8.5) was added, and the reaction was continued overnight at room temperature. Next day, the product was collected by an external magnetic field and the template was washed out 200 µl of solution containing mixture of 5% acetic acid and 1% SDS three times and 200 µl of MilliQ water one time.

Fluorescent microscopy

An Olympus IX71 inverted fluorescence microscope was used for imaging of MIPs/NIPs. The used objective was LUCPLFLN 20 X PH. Total magnifications was 200 \( \times \). Detector of emitted light was Hamamatsu CCD ORCA-HR (C4742-95-12HR) with pixels of 1600 \( \times \) 1200, exposure time was 4 s. Filter was TX Red (\( \lambda_{ex.} \) 545 nm, \( \lambda_{em.} \) 610 nm, dichroic mirror 600 nm). The images of MIPs/NIPs were evaluated using the BRUKER Molecular Imaging software.

Selectivity of adsorption

In the selectivity adsorption experiments, *Enterococcus faecalis* (EF) was chosen as a competitor. It is gram-positive bacteria and that has very similar size and shape as SA. The experiment was carried out as follows: to the MPs-MIPs were added 200 µl of EF or SA (1 \( \times 10^6 \) CFU/ml) suspended in 20 mM TRIS (pH 8.5) and mixture was shaking for 1 hour. Then the unbound bacteria were taken away and MPs-MIPs were washed by 200 µl of MilliQ water. 18 µl of sample (MPs-MIPs with bound analyte or MPs-NIPs) was mixed with 2 µl of 50 µM SYTO 9 (dissolve in dimethylsulfoxide DMSO) and the mixture reacted for 10 minutes. Then the mixture was determined by fluorescence microscope (\( \lambda_{ex.} \) 545 nm, \( \lambda_{em.} \) 610 nm, dichroic mirror 600 nm).

RESULTS AND DISCUSSION

Molecularly imprinted polymers have same function as biomolecules that are very often used for bacterial detection but are much cheaper and stable. Therefore, use of molecular imprinting technology as a promising road to overcome these problems was suggested. In this work, an approach where the molecularly imprinted layer was prepared on the surface of MPs was chosen. A scheme of MIPs preparation process is shown in Figure 1.
Figure 1 Scheme of isolation and detection of bacteria *Staphylococcus aureus* by magnetic molecularly imprinted polymers. A) The templates (bacteria) are mixed with magnetic nanoparticles and functional monomer (dopamine). B) Created MIPs are used for isolation of analytes (bacteria) from sample. Bounding bacteria was stained by fluorescent dye (SYTO 9) and determined by fluorescence microscopy (ex. 545 nm, em. 610 nm, dichroic mirror 600 nm)

**Verification of removal step**

Before the prepared imprinted materials can be used in chosen application, the template molecules (bacteria) have to be removed from the polymer layer.

Prepared MIP surface has to be exhaustively washed to properly remove the template remaining within the polymerized layer to lower the background signal. Therefore, template removal is essential for method validation because this can lead to binding sites damage in terms of collapse or change in shape or size (e.g. polymer swelling).

Conditions introduced in previous work were used for template removal (Hutarova et al. 2017). Briefly, MPs-MIPs/NIPs were washed three times by 200 µl of mixture of 5% HAc and 1% SDS in ratio 1 : 1 and then once by 200 µl MilliQ water. For verification of these conditions, MPs-MIPs and MPs-NIPs before removal step and after removal step were measured. MPs-NIPs are prepared under identical conditions as MPs-MIPs. The difference is that MPs-NIPs are prepared without presence of template. MPs-NIPs are used as an indicator of nonspecific interactions with polydopamine layer. From obtained data (Figure 3 A), it can be seen that during removal step, 95% of template were washed out from MPs-MIPs surface. Therefore this approach was chosen as suitable.

**Binding properties**

The binding properties of prepared MPs-MIPs were investigated using $1 \times 10^6$ CFU/ml of model bacteria (SA) comparing the binding yield of MPs-MIPs and MPs-NIPs. In Figure 2, fluorescence micrographs of MIPs and NIPs prepared for SA (ex. 545 nm, em. 610 nm, dichroic mirror 600 nm) are shown. It was observed that the adsorption of bacteria on MPs-MIPs is significantly higher than in case of MPs-NIPs. The interaction of the analyte (i.e. SA) is lower with MPs-NIPs due to the unorganized and non-specific interaction between the surface and the analyte.

Data obtained using fluorescence microscopy were subsequently processed by BRUKER Molecular Imaging software. This software is able to evaluate the intensity of all fluorescent points and summarize it. This data is more suitable for quantification of efficiency of prepared imprinted materials (Figure 3).
Figure 2 Micrographs of MPs-MIPs (A) and MPs-NIPs (B) with bacteria SA (1 × 10^6 CFU/ml) stained by fluorescent dye SYTO 9

Selectivity of adsorption

For finding of selectivity it was necessary to measure the binding properties of created MPs-MIPs with a competing analyte. In this work, *Enterococcus faecalis* (EF) was chosen as the competitor. These gram-positive bacteria have very similar size and shape as SA.

Measurements were repeated three times and resulting value is an average value of these three measurements. The obtain data are shown in Figure 3 B. From obtain data it is seen that on the surface of MPs-MIPs, prepared for selective detection of SA, 65% larger amount of SA than competitor (EF) was detected. By this experiment, the selectivity of developed MPs-MIPs was confirmed.

Figure 3 A) Quantification of template removal step (MIPs/NIPs were washed out by mixture of 5% HAc and 1% SDS in ration 1 : 1 three times and then once by 200 µl MilliQ water) B) Cross-adsorption of the magnetic imprinted nanoparticles (EF was used as a competitor of interaction)

CONCLUSION

In this work, the MIPs selective for *Staphylococcus aureus* were prepared via a technique of non-covalent molecular imprinting. Dopamine was used as a functional monomer. Experiments focused on molecular recognition efficiency of created polymers were carried out. The resulting imprinted polymers demonstrated great binding properties and high selectivity under the optimal conditions. Moreover, this method is very simple, specific, low cost and eco-friendly. Future work will be focused on isolation of bacteria from real sample for example from milk. SA is the bacteria that causes range of serious infections and is a threat to public health. Therefore, the Legislation of EU has set limits that cannot be exceeded in commercially available foods. The presented technique enables fast and easy method could find potential in food industry.
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REFERENCES


