Porous Silicon modified by Molecularly Imprinted Polymers for Interleukin-6 (IL-6) detection.

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Abstract. In this study, we present an innovative optical biosensor designed for the precise detection of Interleukin-6 (IL-6), a crucial cytokine associated with various pathological conditions. Our biosensor is based on silicon porous material meticulously modified with a molecularly imprinted polymer (MIP), ensuring specific and sensitive recognition of IL-6 molecules. Fabrication process involves the electrochemical etching of silicon porous chips followed by the electrodeposition of MIP, tailored to selectively bind IL-6 targets. Through rigorous testing across a range of IL-6 concentrations, our sensor exhibits remarkable sensitivity, showcasing discernible optical responses proportional to the varying analyte concentrations. Furthermore, we assessed the sensor's performance using bovine serum, a complex biological matrix, to simulate real-world sample conditions. Encouragingly, the sensor maintains its selectivity and optical response in the presence of serum components, affirming its robustness and applicability in practical diagnostic settings.

1 Introduction

Biosensors, leveraging transducer elements to convert biological information into easily processed signals, have emerged as indispensable tools across various biomedical applications [1]. Among these transducers, porous silicon (PSi) stands out for its remarkable properties, including air-filled pores, a large surface area, and tunable surface chemistry. These characteristics render PSi an attractive platform for biosensing applications, particularly in optical biosensors, owing to its ability to generate sharp optical resonances and accommodate large wavelength intervals. Moreover, the high surface reactivity of PSi enables the efficient immobilization of biomolecules within its porous matrix, facilitating the sensitive detection of target analytes[2]. In the quest of enhancing biosensor performance, the integration of polymers with PSi devices has shown considerable promise by offering a multitude of advantages such as biocompatibility, conductivity, and the ability to introduce new functionalities for molecular recognition, thereby augmenting sensor selectivity and sensitivity[3]. Among polymer-based strategies, molecularly imprinted polymers (MIPs) have garnered significant attention as synthetic receptors. MIPs offer advantages such as enhanced stability, cost-effectiveness, and rapid fabrication compared to natural biomolecules [4]. In this study, we use the capabilities of PSi and MIP technology to develop a novel optical biosensor tailored for the detection of Interleukin-6 (IL-6), a pivotal cytokine associated with various diseases [5]. While traditional detection methods often rely on costly and time-consuming immunoassays, our approach offers a rapid, sensitive, and cost-effective alternative suitable for point-of-care diagnostics. By electro-polymerizing the functional monomer o-phenylenediamine (o-PD) in the presence of IL-6, we synthesized an MIP-based artificial receptor specifically designed for IL-6 recognition [4]. Our three-step process, encompassing polymerization, template removal, and rebinding, demonstrates the sensor's remarkable affinity and specificity for IL-6 detection. This integrated approach represents a significant advancement in biosensing technology, offering a versatile and efficient platform for the rapid analysis of IL-6 in clinical settings.

2 Methods

The single-layer porous silicon (PSi) structure was created through an electrochemical etching process of n-type crystalline silicon. This etching process occurred in a solution consisting of 200 mL of 5% HF in weight mixed with ethanol at room temperature (RT. The resulting PSi monolayer exhibited a pore size distribution ranging from 50 to 250 nm. This fabrication was achieved by applying a current density of 20 mA cm^{-2}. After chip fabrication, it was stored under argon to prevent oxidation. After, the electrosynthesis of Po-PD was conducted on a PSi substrate, utilized as the working electrode, through cyclic voltammetry (CV). This process occurred in a solution of acetate buffer (0.5 M, pH 5.2), containing 0.1 mg/mL of

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o-PD. Before the polymerization, IL-6 was incorporated into the o-PD solution as a template molecule.

3 Results and Discussions

In this study, the detection of Interleukin-6 (IL-6) was achieved using a porous silicon platform modified with molecularly imprinted polymers (MIP). To comprehensively evaluate the sensor's performance, various concentrations of IL-6 dissolved in phosphate-buffered saline (PBS) were tested. Following the electro-deposition of the polymer, the chip underwent an acetic acid wash to remove excess polymer. This step was characterized by reflectance measurements, revealing a noticeable blue shift in the spectrum post-washing, indicative of successful polymer removal and efficient IL-6 binding sites exposure [6]. Subsequently, IL-6 protein was incubated on the chip in PBS for one hour, followed by washing and measurement of its reflectance signal. Notably, increasing concentrations of IL-6 led to larger shifts, demonstrating a robust linear relationship with IL-6 concentrations in the nanomolar range.

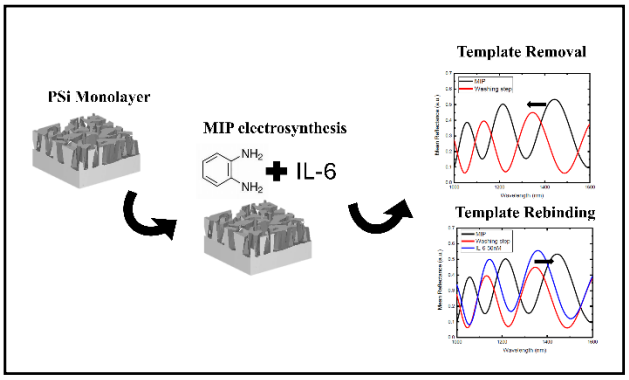

Fig. 1. Fabbrication scheme of PSi modified by MIP-electrosynthesis. Reflectance spectra of (IL-6) template removal and (IL-6) template rebinding.

These results underscore the effectiveness of the developed sensor for sensitive IL-6 detection, highlighting its potential significance in biomedical research and diagnostics. Additionally, specificity experiments were conducted to assess the sensor's ability to selectively recognize IL-6 in the presence of other proteins. This was achieved by incubating transforming growth factor-beta (TGF-β) protein on MIP-modified porous silicon and IL-6 on non-imprinted polymer (NIP)-modified porous silicon. The results indicated the sensor's capacity for selective recognition of IL-6. Furthermore, the sensor's performance was evaluated in a more complex matrix, bovine serum, to mimic real-world biological samples. Encouragingly, the sensor maintained its specificity in the selective recognition of IL-6 even in the presence of additional proteins. These comprehensive experiments confirm the versatility and potential applicability of the sensor across diverse biological samples, underscoring its reliability for targeted cytokine detection in complex environments.

4 Conclusions

In conclusion, the developed biosensor, utilizing molecularly imprinted polymers (MIP) integrated with porous silicon (PSi) for IL-6 detection, demonstrates significant promise for biomedical applications. Through meticulous optimization and thorough testing, the sensor exhibits remarkable sensitivity and specificity in detecting IL-6 across a range of concentrations. Its ability to selectively recognize IL-6, even in the presence of other proteins, underscores its potential for use in complex biological matrices such as serum. Furthermore, the sensor's compatibility with real-world samples enhances its applicability for diverse diagnostic scenarios.

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