Biosensors and Bioelectronics 50 (2013) 425–430

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Point-of-care protein sensing platform based on immuno-like membrane with molecularly-aligned nanocavities

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ARTICLE INFO

Article history:
Received 26 April 2013
Received in revised form
8 July 2013
Accepted 9 July 2013
Available online 17 July 2013

Keywords:
Immune-like membrane
Protein biosensor
Microfluidic biochip
Point-of-care platform

ABSTRACT

This paper presents the ability of the novel point-of-care protein sensing platform based on immune-like polymer membrane to separate and sense target analytes in human serum samples using the molecularly-aligned nanocavities. The separation performance of the developed membrane, which is substantially affected by surface chemistry and physics, can be enhanced by alignment of the template molecules. The developed biomimetic membrane with aligned molecular nanocavities can be synthesized and integrated with microfluidic biochips as point-of-care sensing platforms. The measurement results showed that the specific adhesion forces of the developed highly-aligned nanocavities on the immuno-like membranes are comparable to the interaction forces between CRP and biological CRP antibodies. The biomimetic polymer membrane works as antibody to catch specific proteins in complex biofluids within 110 s. The proposed approach is an adaptive technological platform because it facilitates cost-effective mass production and can be applied to a wide range of protein biomarkers.

1. Introduction

Traditional instruments requiring large fluidic samples, complex and lengthy procedures, and professional operators are widely used in clinical laboratories for disease diagnostics. Point-of-care assays based on microfluidic biochips can substantially improve the quality of medical care because of their ease of access, fast response, small bio-sample volume, and portability. A biological enzyme or antibody is the most popular molecular recognition layer for biosensors. Most biochips use biological antibodies with labels for the molecular recognition layers for the optical detection of the target molecules. However, biological antibodies must be stored under −20 °C before use. The affinity may change with time when exposed at room temperature. This reduces the compatibility of the fabrication process to other factors, such as the solvent, process temperature, and interface, for molecular immobilization. In addition, it causes issues with chip integration and storage, which degrades the binding affinity of the antibodies at room temperature, increases uncertainty, limits storage life, and prevents point-of-care applications. Biosensors based on biological molecular recognition techniques are unsuitable for point-of-care clinical diagnostics. Molecular imprinting, referred to as plastic antibody technology, is a novel technique that provides molecular assemblies of desired chemical structures and properties. Based on the concept of "tailor-made," a particular lock is designed to be unlocked by a specific key, mimicking the lock and key model of antigen–antibodies. Molecularly imprinted recognition structures can be artificially synthesized if required. The advantages of such structures include stronger physical and chemical stabilities, long lifecycles, and a low cost. In the presence of a template molecule, functional monomers are polymerized and immobilized complementarily to this molecule. During these processes, a number of functional monomers are assembled in an orderly manner with their functional groups placed at the desired sites within the cavities of the desired size. Therefore, molecular imprinted polymers (MIPs) with binding sites with specific shape and functional group recognition benefit from the high selectivity and high sensitivity sensing in the target molecular compound (Haupt et al., 2000; Bossi et al., 2001).

Biomimetic plastic antibodies based on molecular imprinting are crucial to developing fully integrated microfluidic biochips with biosensors for point-of-care protein testing. As shown in previous studies, molecular imprinted membranes as biosensors have been integrated in microfluidic biochips for detection of small molecules, such as atrazine, glucose, and the propofol anesthetic agent (Piletsky et al., 1995; Malitesta et al., 1999; Hong et al., 2010). Protein imprinted polymers have been examined for the detection of E7 protein

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http://dx.doi.org/10.1016/j.bios.2013.07.016

(Cai et al., 2010). The specificity in morphology on imprinted nanocavities on a molecular scale has not been addressed in previous studies. In molecular imprinting, the target molecule acts as the template around which the interacting and cross-linking monomers are arranged and co-polymerized to form imprinted nanocavities after polymerization and template removal. The morphology and functional groups of binding sites are strongly related to the selectivity of rebinding the target molecule. Thus, developing immuno-like membranes with efficiently-patterned nanocavities is vital for exhibiting high specificity comparable to biological antibodies. An on-chip molecularly imprinted biosensor was developed for a small molecule (propofol molecule). The polymerization process and the morphology specificity of imprinted nanocavities were examined on a molecular scale (Hong et al., 2012). The influence of surface morphology and alignment of imprinted protein templates on specific interaction force of imprinted functional nanocavities at the nanoscale on the biomimetic immuno-like membranes remains unclear.

C-reactive protein (CRP) is a sensitive marker of inflammation and is primarily synthesized in hepatocytes in response to proinflammatory cytokines, such as tumor necrosis factor alpha and interleukin 6, because of various acute or chronic stimuli (Castell et al., 1990). Elevated serum levels are observed after trauma, tissue necrosis, infection, surgery, and organ failure, and are associated with an increased risk of atherosclerosis, coronary artery disease, stroke, endometriosis, or preterm delivery in pregnancy (Meisner et al., 2006; Makita et al., 2005; Karinen et al., 2005; Orre et al., 2011). The methods used for CRP assay in serum or plasma can be quantitative, semi-quantitative, or qualitative depending on the instruments used. Currently, CRP is measured in clinical laboratories using immunonephelometric or immunoturbidimetric assays (Albrecht et al., 2008). In this work, CRP was used to demonstrate the feasibility of the proposed method. In the near future, plastic-antibody-based biochips can be attached to pre-grammed shape memory polymers for automated fluid operation (Hong and Chen, 2011). The potential applications include cancer diagnostics (Stern et al., 2010), protein–drug interaction (Karlsson et al., 2000), isolation of tumor cells (Yu et al., 2011), and food safety monitoring (Glynn et al., 2006).

Biomimetic immuno-like membranes were developed with aligned molecular nanocavities and compared them with imprinted membranes with random orientation and biological antibodies for surface morphology, alignment degree, and specific interaction force. In addition, the developed membranes were integrated into plastic microfluidic biochips for rapid and precise measurements of CRP in human serum samples (Fig. 1). MIPs for a macromolecule

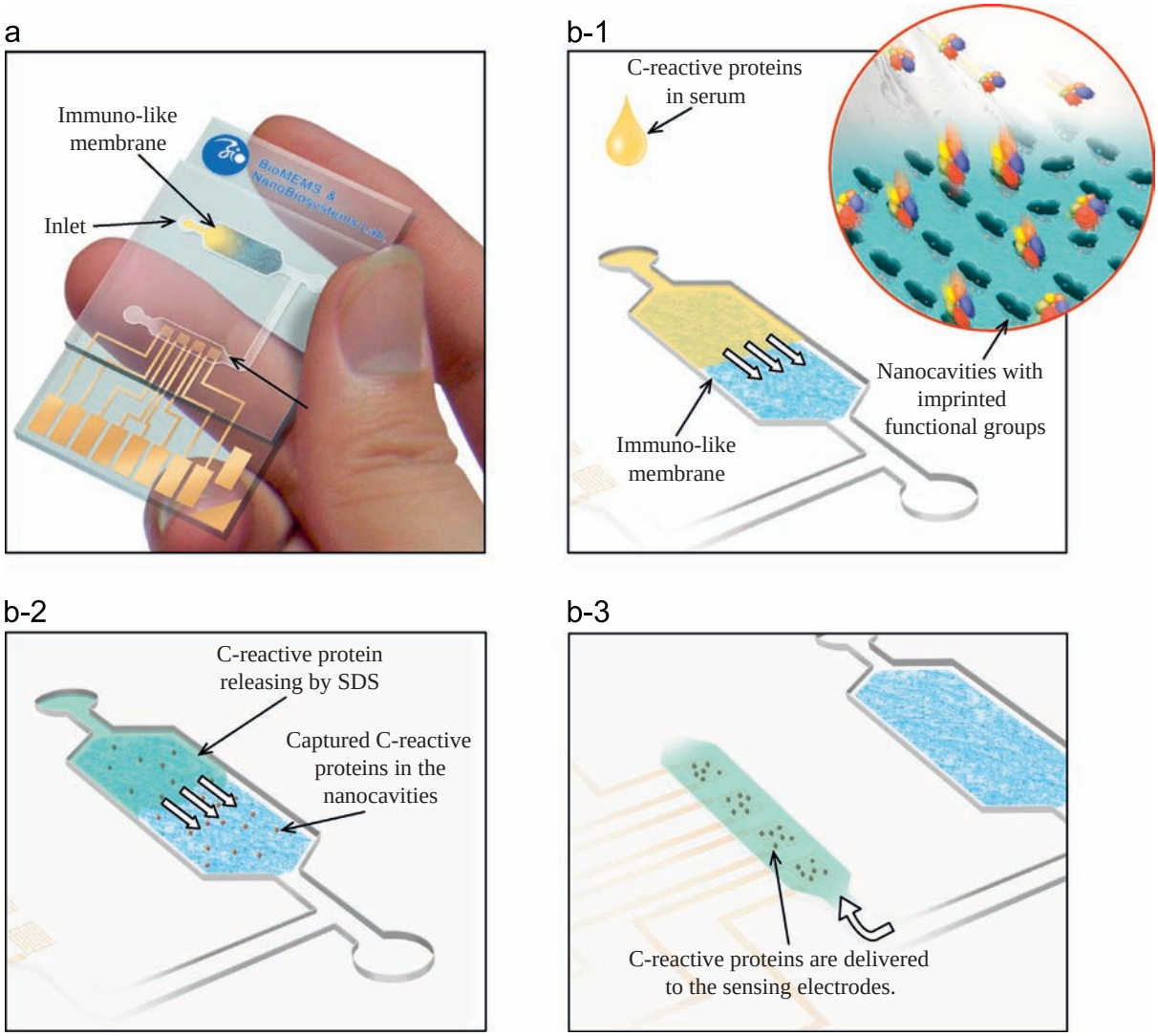

Fig. 1. Schematic illustration of the proposed biomimetic immuno-like membranes with aligned molecular nanocavities for point-of-care protein biosensing. (a) Immuno-like membrane in microfluidic biochips, (b) separation and sensing procedures, (b-1) loading of human serum samples into the microfluidic biochips and capturing CRPs from human serum samples by the immuno-like membrane, (b-2) loading of SDS and releasing of CRPs from the immuno-like membrane, and (b-3) delivery of SDS with CRPs to the electrodes for electronic sensing.