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DNA-directed antibody immobilization for universal biothreat agent detection in the field

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Abstract

Natural outbreaks and the willful use of biothreat agents for acts of terror demonstrate the need to immediately detect and identify biothreat agents. The portable immunoassay based biochip platform pBDi was shown to be well suited for a very sensitive and fully automated detection of biothreat agents. However, one challenging aspect of protein microarray production is printing the proteins onto the sensor surface. Here, we demonstrate an alternative solution for site specific immobilization of antibodies using DNA-directed immobilization (DDI) and the usage of in this way generated biochips for sensitive detection of biothreat agents. In DDI, each capture antibody is encoded by a specific DNA sequence covalently attached to it. The antibodies immobilize on their complementary DNA probes immobilized on the gold electrode surface via sequence specific DNA/DNA hybridization. This immobilization approach has several advantages over direct covalent attachment of antibodies, such as increased availability of binding sites for analyte capture by decreasing the steric hindrance and more favorable orientation of antibodies. Exemplary, we present an addressable biochip carrying antibodies specific for *F. tularensis*, *orthopox* virus and the plant toxin ricin. Limit of detection (LOD) for *F. tularensis* and *vaccinia* virus is approx. 10^3 CFU mL^{-1} or 10^4 PFU mL^{-1}, respectively, whereas LOD for ricin is 0.1 ng mL^{-1} applying an analysis time of approx. 25 minutes. Furthermore, we demonstrate specificity of the addressable biochip and detection of biothreat agents in food and environmental matrices.

Introduction

Due to the potential risk of terrorist attacks using highly toxic or pathogenic biothreat agents there is a need for rapid, sensitive and specific detection of all kinds of biothreat agents (bacteria, viruses and toxins) in the field.

Methods

pBDi platform:

- Detection platform integrated in a robust suitcase for use in the field by first responders (Fig. 1 A).
- Capture antibodies against specific biothreat agents are immobilized on electrodes of a biochip (16 interdigitated gold electrodes) via DNA/DNA hybridization (Fig. 1 B).
- Based on an automated electrochemical sandwich-ELISA (Fig. 1 C).
- Multiplex detection of 3 biothreat agents within 25 minutes.
- Inherent positive and negative control.
- Very sensitive due to strongly amplified electrochemical detection system including a redox cycling process of enzyme product between interdigitated electrode structure (Fig. 1 C).
- Fully automated fluidics.
- Software controlled data analysis.

Preparation of antibody-DNA conjugates:

The antibody-DNA conjugates were synthesized by coupling an amino modified oligonucleotide to an antibody, in which the lysine residues were nonspecifically modified with hydrazine (according to Antibody-Oligonucleotide All-in-One Conjugation Kit, Solulink, Inc.). Antibody-DNA conjugates were characterized by SDS-PAGE and South-Western blotting.

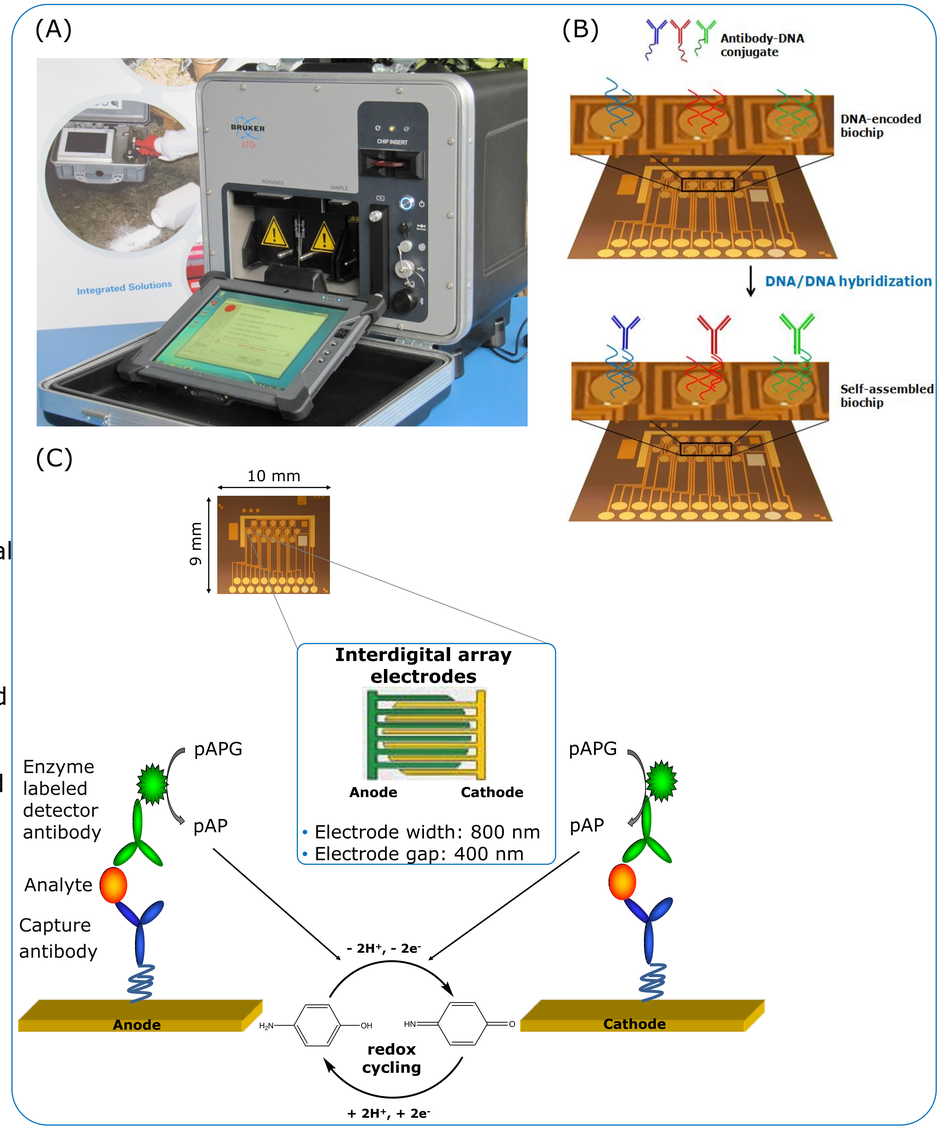

Fig. 1 (A) The pBDi platform. (B) Principle of electrochemical sandwich immunoassay. (C) Principle of DNA-directed immobilization of antibodies [modified from Elsholz *et al.* (2006), Anal Chem 78:4794-4802].

Results

- Design of an addressable biochip using antibody-DNA conjugates based on pBDi platform.
- Biothreat agents detection with pBDi together with an addressable biochip shows excellent limits of detection (LODs) (Tab. 1).
- Addressable biochip allows specific detection of biothreat agents (Fig. 2).
- Detection of *F. tularensis* in different sample matrices is demonstrated (Fig. 3).

Antigen	LOD	
	Random orientation	DDI
F. tularensis	$5x10^4$ CFU mL^{-1}	$5x10^3$ CFU mL^{-1}
Vaccinia virus	$9x10^4$ PFU mL^{-1}	$9x10^3$ PFU mL^{-1}
Ricin	0.3 ng mL^{-1}	0.1 ng mL^{-1}

Tab. 1 Summary of LODs obtained with DDI in comparison to random immobilization of antibodies.

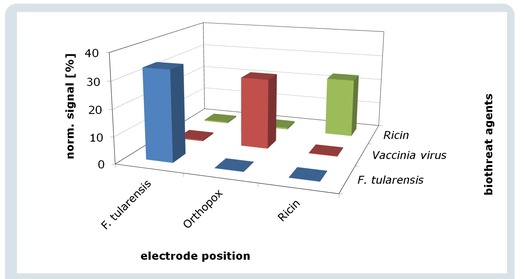

Fig. 2 Specificity of microorganism detection. Application of $5x10^6$ CFU mL^{-1} *F. tularensis* (heat inactivated), 10^7 PFU mL^{-1} *Vaccinia* virus (beta-propiolactone inactivated) and 100 ng mL^{-1} ricin.

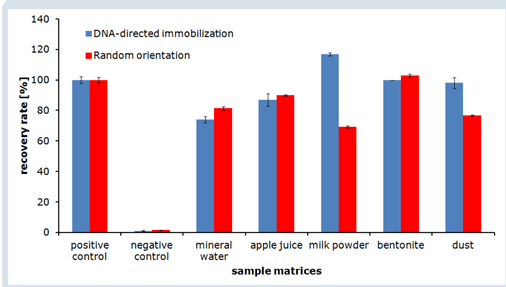

Fig. 3 Detection of $5x10^4$ CFU mL^{-1} heat-inactivated *F. tularensis* in various liquid and solid sample matrices.

Summary

DNA-directed immobilization of antibodies allows the generation of addressable biochips offering rapid and sensitive detection of different biothreat agents. Application of DDI resulted in improved assay sensitivity compared to conventional immobilization procedure. Antibody-DNA conjugates in conjunction with the pBDi platform offer a great potential for the design of customer-specific biochips.

Conclusions

- Oriented immobilization of antibodies via DDI
- Excellent sensitivity
- Simultaneous detection of bacteria, viruses and toxins
- Open platform technology with maximal flexibility

pBDi

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