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Ligands for label-free detection of whole bacteria on biosensors: A review

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ABSTRACT

With the aim of getting earlier, sensitive and specific information on the presence –or absence – of bacterial pathogens, biosensors are getting an increasing interest for more than two decades. This is partly due to their reduced format, to the possibility to address several questions with a single device and also to the increasing panel of physical approaches that can be exploited for signal transducing. When designing a biosensor, the choice of the ligand motif remains a key element as it drives the efficiency and sensitivity of the assay. In this review, we propose to gather and comment different ligands used for the detection of whole cell bacteria. Because time is a crucial issue when looking for a pathogen, our attention was focused on whole cell assays and label-free methods, which enable the user to skip sampling processing steps and decrease the overall test cost.

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1. Introduction

From our skin to our plates, going through all our electronic devices, such as phones and computers, microbes are everywhere around us. Although most bacteria are harmless, a few can cause various diseases ranging from minor incidence to lethal issues. Among the food-borne pathogens, *Salmonella*, *Listeria monocytogenes* and enterohemorrhagic *Escherichia coli* are responsible for several millions of diseases worldwide each year [1]. The World Health Organization will soon published a report estimating the Global Burden of Foodborne Diseases (http://www.who.int/foodsafety/foodborne_disease/ferg/en/ accessed on September 30, 2015). In hospital settings, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are a considerable source of nosocomial infections. To

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efficiently fight these germs and reduce their impact on human health, researchers and physicians need to quickly recognize them.

Nowadays, bacteria detection still relies for the most part on classical microbiology methods including isolation and growth in selective media [2]. Although proven to be efficient, these techniques are labor intensive and time consuming due to the growth phase needed for microorganism development. It is therefore crucial to design new and innovative bacterial detection methods. To that aim, modern technics, including mass spectrometry, microarray, PCR and genomic sequencing have been intensively investigated [3]. Nevertheless, these methods generally require high technical skills, intense sample processing and rely on the presence of bacterial molecules instead of whole bacterial cells.

Over the past years, the development of biosensors has also been the focus of exhaustive researches. A biosensor is an analytical device converting a biological response into a measurable signal [4]. It is generally composed of three elements: (1) a ligand grafted on the biosensor surface which recognizes a target through specific interactions. It needs to be specific and sensitive against its target in order to induce a positive signal and prevent the interference by other substances from the sample, (2) a transducer which converts bio-recognition events arising at the surface to a physically quantifiable signal, being classified as electrical, optical, calorimetric, piezoelectric, acoustic or magnetic, (3) a detector which amplifies and analyzes the signal. Thus, biosensors convert a bio-recognition event into a physically measurable signal. The Fig. 1 gives an outline of the ligands described in this review.

Ideally, these devices need to be small, portable, easy-to-use and able to work at the point-of-care, in contrast to other diagnostic techniques. Furthermore, in order to be adapted to pathogen detection, biosensors have to give highly reproducible and rapid results. The choice of the bioreceptor is crucial for the efficiency of the biosensor, as it can influence both its sensitivity and specificity for the bacterial target. To date, a broad spectrum of bioreceptors has been used for bacterial detection. In this review, we propose an overview of the principal ligands used in biosensor systems for label-free detection of whole bacterial cells. These crucial probes are classified into three categories depending on their origin. Some ligands are natural products, or derivatives of natural products; others are engineered bio-molecules inspired from natural products whose properties are artificially improved; and eventually, some ligands are randomly synthesized biomolecules whose natural affinity for a target enables their selection from large molecular libraries. Label-free detection enables real-time measurement of the interaction and consequently requires less time and reagents than label-based methods. We will focus on whole bacteria detection, which can be directly applied on samples without the need of sample processing prior to the analysis.

2. Natural ligands

2.1. Antibodies

Antibodies (Abs), or Immunoglobulins (Igs), are host proteins produced by the immune system of eukaryotes to neutralize and eliminate pathogens. These “Y shape” proteins are typically composed of four chains, two large heavy chains (V_H) and two small light chains (V_L). They possess two distinct regions, the fragment crystallizable region (Fc fragment) which interacts with and activates other immune system partners and the antigen-binding region (Fab fragment) that recognizes and binds to antigenic agents through a specific recognition domain called epitope. With the need for modern and rapid biosensing systems, Abs have become key affinity ligands for pathogens detection in food and clinical samples [5]. Indeed, Immobilized Abs can interact with antigens on microbial surfaces, inducing a measurable signal by an output detector. Their popularity arises from several advantages, such as versatility, ease of integration into different systems and high specificity toward their targets.

Abs production is generally based on the injection of inactivated whole microbes, surface or soluble components into a suitable animal host, such as mice, rabbits, goats, horses or sheeps. Most antigens are highly complex and present several epitopes on their surface. Therefore, the immune response to an antigen generally involves the activation of multiple B-cells all of which targeting a specific epitope. As a result, various antibodies with different epitope specificities are produced. These are known as Polyclonal Antibodies (PAbs). In contrast, Monoclonal Antibodies (MAbs) are produced by a single B cell and contain a pool of the same antibody that binds

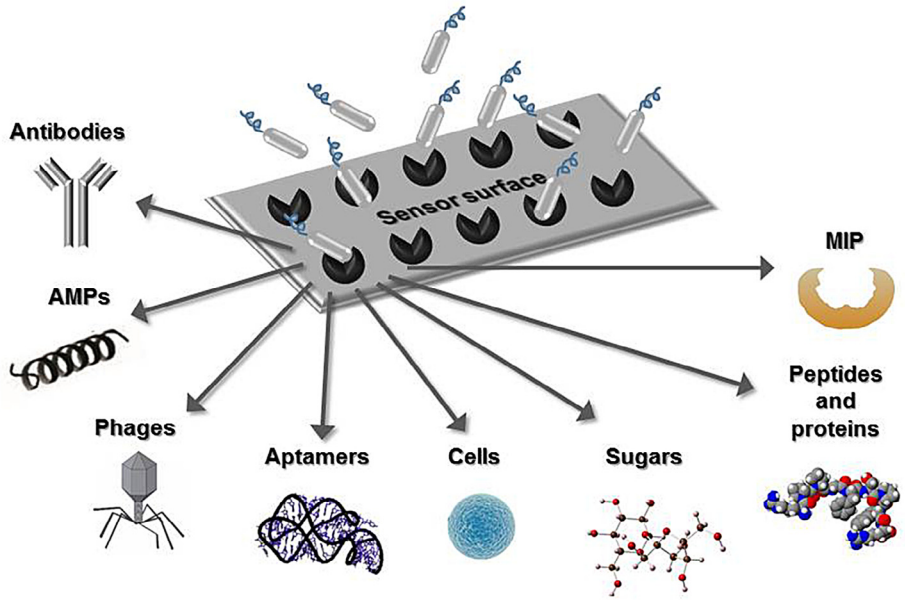

Fig. 1. Overview of the different ligands integrated in biosensing platforms.