



Facing the Challenges of Pharmaceutical Research Using Whispering Gallery Modes-Based Biosensors

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Abstract

During the last decade, different Whispering Gallery Modes-based biosensors (WGMS) have been proposed as bioanalytical platforms. These systems allow for a label-free real-time monitoring of a wide range of molecules, including DNA, proteins and polymers. With the advancement of new technologies and the increasing complexity of novel biotherapeutics, the development of versatile tools is of great relevance to meet the needs of the pharmaceutical research. Throughout this review, the role of WGMS in the drug development process and their ability to face the current challenges and limitations in this field will be discussed.

Keywords: Whispering Gallery Modes, Biosensors, Label-free detection, Drug development

Abbreviations: WGMS: Whispering Gallery Modes-based Sensors, SPR: Surface Plasmon Resonance, KA: Association Constant, KD: Dissociation Constant

Introduction

Optical biosensors are well-known analytical tools for probing biomolecular interactions between molecules. These sensors have a wide range of applications including medical diagnostics (e.g. cancer biomarker detection) and drug development [1]. In the last years, there has been a growing interest for label-free technologies, which present intrinsic advantages over their label-based counterparts, including real-time kinetic analysis, faster development and no bio-affinity alteration due to the incorporated label [2]. There are several label free technologies, including Whispering Gallery Modes-based biosensors (WGMS). The basic principle of WGM relies upon total internal reflection of light trapped inside a circular resonator which circulates along the surface, producing associated photons with certain wavelengths which can be detected as narrow peaks in the optical spectrum [3]. Any modification in the optical geometry or refractive index on the surrounding environment (e.g. binding of molecules to the resonator surface) induces a shift in WGM wavelength positions, which can be correlated with the total number of bound molecules. Due to the small diameters of the circular resonators between 200 and 7 μm , they can detect

less molecules and can be used in miniaturized devices [4]. The specificity for the analyte detection relies on surface immobilized molecules (e.g. antibodies, membrane receptors) which react selectively with the analyte of interest. Several strategies have been described for surface immobilization, being either random or site-directed (e.g. by protein A, G) [5].

Use of WGMS in (pre)clinical drug development

One critical parameter that needs to be assessed during the drug development process is the binding profile of biotherapeutics to their target molecule (e.g. association (KA) and dissociation (KD) constants). An example of WGMS for determining the KD of the biotin-streptavidin binding pair was reported by Soteropolus et al. [6]. In this study, the KD was estimated from a linearization of the dissociation phase in good agreement with previous values in the literature. However, efforts need to be made to reduce mass transport limitations problems, for example by optimizing the flow rate and ligand concentration, for an accurately determination of the KA. Additionally, for kinetic analysis each binding pair

should be tested empirically to evaluate the most suitable site-directed immobilization method in order to control the density of binding sites on the sensor surface, as very high one might alter the KD and KA values. On the other hand, the increasing complexity of novel biotherapeutics has raised new challenges for functional characterization, as when compared to standard antibodies, bispecific antibodies require the consideration of two individual interactions [7]. Only a couple of technologies, including suspension array technology [8] or surface plasmon resonance (SPR) have been described for bi-functional characterization in one approach. Meschendoerfer et al. have proposed a SPR-based dual binding assay in which a bispecific antibody is immobilized on the SPR chip through a capture antibody [9]. Despite their promising results, the capture antibody introduces an additional interaction that is not totally desirable. While current SPR systems are ideal for characterizing single interaction pairs, they might expertise difficulties resolving multi-variant interactions due to the limited number of available configurations and the lack of controlled immobilization of ligands on the sensor surface [10]. To the best of our knowledge, WGMS haven't been described for bi-functional characterization although their versatility for surface immobilization of molecules on the sensor surface and the possibility of using geometrical cell-like-microparticles as sensors, offering a more 3D realistic interaction setting, makes this technology an attractive alternative for functional analysis of new bi-functional therapeutic agents.

Use of WGMS for real-time monitoring of therapy response

Another important stage in the drug development process is the *in vitro* assessment of cell responses after drug treatment. Chen et al. described in 2016 a WGMS for the specific and label-free detection of procaspase-3 released from human cell lines after treatment with a toxin [11]. The same group has recently improved the system by integrating WGMS with a microfluidic platform for detecting cytochrome c, a soluble biomarker of apoptosis, with nanomolar sensitivity in cell culture media after treatment of cell lines in the same way [12]. Although several parameters such as cell culture perturbation or the limit of detection still need to be fully assessed, these initial studies suggests a potential role of WGMS for the non-invasive real time monitoring of biomarkers release from intact and nonlabelled cell cultures, which might be also implemented within complex organ-on-a-chip systems.

Conclusion

Some of the most important challenges and applications of WGMS within the biopharmaceutical research have been

discussed. Although more efforts need to be done to improve WGMS competitiveness, WGMS are a worthy technology to look at for facing the needs of the biopharmaceutical and biotechnological research and overcome some of the limitations of current methods.

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