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Quartz crystal microbalance: Sensing cell-substrate adhesion and beyond

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ABSTRACT

Cell adhesion is an essential aspect of cellular behavior. Finding innovative methods to probe the adhesion of cells in their native state can greatly advance the understanding of control and regulation of cellular behavior and their impact on human health. The quartz crystal microbalance (QCM) is a label-free, biosensing system that has, in the past fifty years, evolved from a simple acoustic based mass sensor to a powerful bioanalytical tool. Its unique capability of monitoring the cell-substrate interaction non-invasively in real time has led to the emergence of its applications in areas that are relevant to fundamental cell biology and medical research. This review is intended to provide readers an overview of the use of the QCM for examination of cell-substrate adhesion. It also describes how this innovative approach can be extended to the study of other aspects of cellular behavior, such as cell morphology, cell mechanics, cell motility, cell signaling, all of which can potentially be applied to medical diagnosis and/or pharmaceutical development. In this review a major emphasis is placed on informing readers about some of the most important practical aspects of the QCM-based cell study including data acquisition and analysis, the substrate surface manipulation, and cell manipulation.

1. Overview

Cell adhesion is essential for fundamental cellular processes such as cell proliferation, migration, differentiation, and survival (Gumbiner, 1996; Huang and Ingber, 1999). Adhesion, which is established through the engagement of cell membrane receptor integrin with extracellular matrix (ECM), provides a structural and functional connection between the intracellular and the extracellular environments (Juliano, 2002). Abnormality in cell adhesion is a cellular disorder associated with a wide range of human diseases, such as cancer (Bendas and Borsig, 2012; Cavallaro and Christofori, 2001), arthritis (Morel et al., 2002; Veale and Maple, 1996), atherosclerosis (Blankenberg et al., 2003; Galkina and Ley, 2007), and osteoporosis (Miura et al., 2005; Perinpanayagam et al., 2001). A comprehensive understanding of the development and progression of such a cellular disorder is essential to early diagnosis and successful treatment of these diseases.

Traditionally cell adhesion has been evaluated by means of cell counting and activity assays. The activity assays report the response of adhered cells to specific cellular events, such as gene expression, generation of secondary messengers and/or translocation of labeled targets (Zhang and Xie, 2012). These assays, which usually require steps such as cell lysis, labeling of target protein molecules, and/or fixation, all of which can cause cell death, are considered end-point detection and are unable to provide an assessment of the process of cell adhesion as a function of time. In addition, labeling reagents, such as fluorescent dyes and magnetic particles, may often create non-native and physiologically irrelevant cellular environments, which have the potential to affect the response of the cell (Abbitt et al., 2000; Denholm and Gerald, 1995; Xi et al., 2008). Furthermore, neither counting the numbers nor assaying the activities of the cells is capable of providing information on the mechanical strength of cell-substrate adhesion, which is a critical parameter for assessing structure and function of the cell (Geiger and Yamada, 2011). Mechanical properties of adhered cells are often determined by means of centrifugation, hydrodynamic assays, atomic force microscopy, optical tweezers and traction force microscopy (Dembo and Wang, 1999; Khalili and Ahmad, 2015). However, most of these approaches are considered invasive, simply because external forces are applied to the cell, and these forces can compromise the cell physiology and alter the cell adhesion.

The quartz crystal microbalance (QCM) is a non-invasive, label-free, acoustic sensing system that is capable of real-time monitoring of the adhesive interaction between cells and the surface of the sensing element. Over the past two decades such a unique capability has made the QCM particularly attractive in the field of cell biology (Chronaki et al., 2016; Elmlund et al., 2015; Fohlerová et al., 2007, 2012; Heitmann et al., 2007; Janshoff et al., 1996; Wegener et al., 1998; Zhou et al., 2000). The present review is intended to provide readers an overview of the use of the QCM for examination of cell-substrate adhesion. It also describes how this innovative approach can be used as

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a platform for the study of other aspects of cellular behavior. First in this review is a brief introduction to the basic principles of the QCM. This leads to a discussion in the second section on the relevance of these basic principles to the study of cell adhesion. Also included in the second is a survey of a variety of QCM-based measurements and their pros and cons for evaluation of cell-substrate adhesion. Third is a discussion of how this innovative approach can be used as a platform for the study of other aspects of cellular behavior, such as cell morphology, cell mechanics, cell motility, and cell signaling. The fourth section outlines some useful strategies for obtaining desirable experimental conditions for QCM-based cell studies. Fifth and finally, the review concludes with a recap of the evolution of QCM and an outlook for the future development of applications and instrumentation of QCM.

2. Basic principle of the QCM technology

The QCM is an acoustic sensing system that has been used in chemical (Cheng et al., 2012), physical (Urbakh et al., 2007), biological (Dixon, 2008; Vashist and Vashist, 2011), and biomedical research (Hunter, 2009; Tagaya, 2015). The acoustic sensing by the QCM is based on a piezoelectric crystal, shaped like a disk that can be set into oscillation in the shear-mode by an electrical current. Metal electrodes are deposited on the opposite faces of the quartz crystal. When an AC current is applied by the electrodes, the piezoelectric quartz crystal undergoes an in-plane shear-mode oscillation, which produces shear waves that propagate perpendicularly to the crystal surface, into the contacting medium (Rodahl and Kasemo, 1996). The frequency of the oscillating crystal is extremely sensitive to the nature of the contacting medium. In 1959, Sauerbrey demonstrated that the change in resonance frequency of the oscillating crystal is directly proportional to the change in mass coupled to the surface of the crystal (Sauerbrey, 1959),

$$\Delta m = -\frac{C}{n}\Delta f_n \tag{1}$$

where Δf_n is the change in resonance frequency at the *nth* harmonic (n = 1, 3, ...), Δm is the mass deposited per unit area of crystal surface, and C is the mass sensitivity constant of the instrument. For a 5-MHz crystal, C is 17.7 ng/Hz·cm^2.

The Sauerbrey equation is valid when the mass of the material coupled to the sensor surface is much smaller than the mass of the quartz crystal, and the adhered material is rigid, elastic and evenly distributed over the sensor surface. However, the Sauerbrey relationship becomes invalid when the adhered material is soft and/or submerged in liquid medium. Such a situation is often seen with applications in biotechnology, biosensing, bioanalysis, etc., because of the soft nature of adhered biomaterials and biomolecules involved in these applications. The damping caused by the viscous character of a contacting soft film or liquid results in a shift in resonance frequency additional to that of the added mass alone, resulting in a deviation from the Sauerbrey relationship. Thus, the frequency shift of a soft film is sensitive not only to the mass of the film but also to the solvent that is associated with or hydrodynamically coupled to the film (Wang et al., 2008). The viscous character of a soft film has been routinely characterized by means of monitoring the change in energy dissipation (ΔD) of the vibrational energy of the oscillating crystal or the change in motional resistance (ΔR) of the oscillating crystal. Energy dissipation factor D is a dimensionless quantity that is defined as,

$$D = \frac{\mathrm{E_{dissipated}}}{2\pi E_{stored}} \tag{2}$$

where $E_{dissipated}$ is the energy dissipated during one cycle of oscillation and E_{stored} is the energy stored in the oscillating crystal. ΔD is determined with analysis of decay of the freely oscillating crystal after rapid excitation (Rodahl et al., 1995; Rodahl and Kasemo, 1996), and ΔR is determined with impedance analysis (Johannsmann, 2008). Since ΔD and ΔR are assumed equivalent (Zhang et al., 2009), they are used interchangeably throughout this review. Overall, technology advancement has made the QCM a state-of-the-art mechanical sensing system, which is capable of providing real-time measurement of changes in both resonance frequency (Δf) and energy dissipation (ΔD) of the material that is coupled to the surface of an oscillating piezoelectric crystal.

3. QCM-based measurements in the study of cell adhesion

A majority of cell studies involving the QCM have been focused on cell-substrate adhesion (Cavic et al., 1999; Dixon, 2008; Heitmann et al., 2007; Marx, 2003; Saitakis and Gizeli, 2011; Şeke and Elçin, 2012; Tagaya, 2015; Wegener et al., 2001), which is typically stabilized by complexes formed between cell surface integrin receptors and the ECM, which is located in the basal region of the cell and also adhered to the quartz crystal surface. It has been determined that stronger cell adhesion is associated with the clustering of these complexes together to form focal adhesions (Juliano, 2002). To probe the cell adhesion, the QCM relies on the penetration of the acoustic wave into the basal plane region of the cell. The depth of penetration (δ) can be determined from the following equation:

$$\delta = \sqrt{\frac{\eta}{\pi\rho f_n}} \tag{3}$$

where η is the viscosity of the liquid, ρ is the density of the liquid, and f_n is the frequency of the nth harmonic. Assuming cells have similar properties to those of water, the 3rd harmonic (n = 3) of the acoustic shear wave would have a penetration depth of approximately 100–150 nm (Le Guillou-Buffello et al., 2011). This range of depth is within the basal region of the cell (Kanchanawong et al., 2010) and allows the QCM to target specifically the cell-substrate adhesion with high sensitivity while remaining insensitive to changes beyond the basal region (Wegener et al., 2000).

In recent years, the application of the QCM to the acoustic sensing of whole cells has become trendy (Khalili and Ahmad, 2015; Xi et al., 2013). This can be attributed to the unique capability of the QCM to provide a real-time assessment of both mass and mechanical properties of cells with high sensitivity and time resolution. In addition, as a label-free technology, the QCM avoids non-native perturbations to cells caused by labels, such as fluorescent dyes and magnetic particles. Furthermore, the shear-mode oscillation of the QCM is considered non-invasive to cells adhered to the sensor surface. This is because the lateral displacement of the surface of the sensor crystal rarely exceeds 1 nm during oscillation (Heitmann and Wegener, 2007).

3.1. Frequency-response (Δf)

Most of the cell studies involving the QCM have been focused on monitoring the adhesion process of various cell types (Da-Silva et al., 2013; Gryte et al., 1993; Kao et al., 2017; Lord et al., 2006; Modin et al., 2006; Redepenning et al., 1993; Tagaya et al., 2011; Wegener et al., 1998, 2000; Westas et al., 2015), during which the resonance frequency of the piezoelectric crystal usually decreased as the result of accumulation of cell mass on the surface of a sensor crystal (Da-Silva et al., 2013; Gryte et al., 1993; Redepenning et al., 1993; Tagaya et al., 2011; Wegener et al., 1999). Those studies showed that the relationship between the change of resonance frequency (Δf) and the change of cell mass (Δm) normally does not obey the Sauerbery equation. This can be explained by the fact that cells behave more like a soft material than like a rigid mass on a QCM sensor surface (Lord et al., 2006; Modin et al., 2006; Wegener et al., 1998, 2000). However, Δf does provide qualitative information about the dynamic process of adhesion. In general, the cell adhesion process begins with an initial stage, where a cell settles onto a substrate to form a very loose physical attachment