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Real-time monitoring of cell mechanical changes induced by endothelial cell activation and their subsequent binding with leukemic cell lines

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ABSTRACT

Endothelial cell (EC) activation and their subsequent binding with different cells have various mechanical consequences that, if monitored real time, can serve as a functional biomarker of many pathophysiological response mechanisms. This work presents an innovative and facile strategy to conduct such monitoring using quartz crystal microbalance (QCM), thereby relating the shifts in its frequency and motional resistance to morphological changes upon cell–cell and cell–substrate interactions. By activating ECs with TNF-α and then characterizing their binding with HL-60 and KG-1 leukemia cells, we are able to induce the mechanical changes in ECs especially in the region of cell–substrate contact which resulted in dynamically coupled mass and viscoelastic changes representing the extent of both activation and binding. The activated ECs suffered a decrease of cellular contact area, leading to positive frequency shift and decreased motional resistance. The binding of leukemia cells onto pre-activated ECs exerted a mechanical force to regain the cell surface contact which resulted in the obvious QCM responses opposite to that of activation, and proportional to the number of cells added, in spite of the fact that these added cells are extremely outside the extinction boundary of the shear wave generated by QCM. Different cell lines demonstrate different attachment behavior, which was detected by the QCM. Despite these variations are quite subtle, yet the sensitivity of the technique for dynamic changes at the interface makes them detectable. Moreover, the reproducibility of the generated data determined at each step by deviation measurements ($<10\%$) in response plot was very high despite the high possible heterogeneity in cell populations. The results are explained on the basis of simple theoretical and physical models, although, the development of a more quantitative and precise model is underway in our laboratory.

1. Introduction

Endothelial cell (EC) activation and its subsequent intercellular interactions can have significant effects on the regulation of various inflammatory responses and can be causative in generating altered microenvironments that play a role in many biological disorders, such as leukemia and drug induced vascular injuries (Oshima et al., 2001; Pezeshkian et al., 2013; Zhang et al., 2010). It has been shown that various factors (e.g. TNF-α, leukemic cells) can induce EC activation (De Assis et al., 2000; Stucki et al., 2001; Woodfin et al., 2009) resulting in altered cell morphology as well as increased expression of various cytokines and cell adhesion molecules (CAMs) such as E-selectin, ICAM and VCAM as shown in Scheme S1 (Carlos et al., 1990; Osborn et al., 1989; Shi et al., 2004). Many of these molecules serve as biomarkers for EC activation (Zhang et al., 2010), with the kinetics of expression directly correlating to the extent of EC activation. Furthermore, these molecules also support leukemic blast and leukemia initiating stem cell (LSC) adhesion to ECs, thereby making a significant contribution in leukemic growth and survival (Hatfield et al., 2006; Li et al., 2010; Li et al., 2004; Salter et al., 2009). We recently demonstrated the direct role of EC activation in promoting chemoresistance of acute myeloid leukemia (AML) cells (Pezeshkian et al., 2013). We found that AML cells were able to induce the activation of resting ECs leading to subsequent adhesion of AML cells through CAMs including E-selectin. Adherence induced a quiescent phenotype that protected AML cells from chemotherapy. Interestingly, adherent leukemia cells could later detach and become

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proliferative, initiating a process resembling relapse. Furthermore, differing leukemia subtypes modulate the EC activation process to varying degrees, which may explain the varied chemotherapeutic responses and relapse rates in different patients. These studies suggest that methods to analyze the propensity of patient specific AML cells to activate ECs may provide an indication of response to therapy and prospectively measure the likeliness of relapse.

Conventionally, EC activation and the subsequently induced cellular interactions (Jin et al., 2006; Sipkins et al., 2005; Wang et al., 2007) are inferred via molecular analyses at the transcriptome or proteome levels with concurrent determination of CAM expression, cell attachment properties, cytokine and chemokine secretion as well as changes in cell morphology and gene expression patterns (Zhang et al., 2010). Unfortunately, most of these methods are time consuming, may require supportive studies using in vivo transplantation in animal models, and provide only retrospective analyses with no real-time information. The quickest method that exists is to measure changes in cell surface expression of biomarker proteins (e.g. CAMs) that are known to be altered during EC activation. Many of these studies are approached using flow cytometry or immunohistochemical staining methods. However, there are two major issues with these approaches. First, the selection of one or even more biomarkers (Zhang et al., 2012) cannot be a true representative of the actual scenario involving multifactor (de Pablo et al., 2013), thus producing misleading results. Even for the selected biomarker proteins, the kinetics of expression may also be different (Duda et al., 2006). Second, numerous biomarkers for EC activation are not considered to be endothelial specific (Pepene, 2012) and can originate from multiple types of cells (e.g. neutrophils, lymphocytes). In order to address these issues, we take a biophysical approach to view EC activation where a population of ECs and the surrounding microenvironment can be considered as an ensemble. EC activation and subsequent adherence of leukemia cells can generate phenotypic alterations in this ensemble, leading to variable cell contacts to the substrate. Thus, by quantifying these mechanical changes, the process of EC activation and the related physiological phenomena can be monitored non-invasively and in real-time. However, the usually employed optical techniques are mostly based on endpoint analysis (Sullivan et al., 2012), thus barring the benefits of this biophysical monitoring. Contrarily, the mechanical phenotyping (Remmerbach et al., 2009) can provide broad scale as well as targeted screening for earlier diagnosis and improved survival rates.

Theoretical description of quartz crystal microbalance (QCM) provided in the Supporting information (SI) indicates that this is one of the best techniques to probe such cellular interactions by relating the biophysical changes in cells to the QCM frequency and energy dissipation. However, the decay length of QCM shear wave is in the nanometer range making it only a surface technique, not able to monitor the cell–cell interactions which are larger in size, e.g. the size of ECs is several microns. But with the described ensemble of cells and their microenvironment, a scenario of mass and viscoelastic changes is created, that can be related to the interaction events of different cells as shown in the pioneering work from Wegener et al. (1998, 2000) and Janshoff et al. (1996) for the adhesion of different cell lines onto the QCM surface. More recently, even the cell surfaces have been modeled for their protein binding and other characteristics (Li et al., 2005) using a similar approach which has also been detailed in some good reviews (Saitakis and Gizeli, 2012). Under these scenarios, QCM can innovatively and quantitatively determine these cellular events. Over the years, Dickert et al. (Jenik et al., 2009a, 2009b; Latif et al., 2013; Seifner et al., 2009) have also used QCM sensors to measure different biospecies, however, by using non-cellular response elements (e.g., molecularly imprinted polymers). Contrarily, we have used ECs by themselves as the response element both for measuring their own biophysical changes during activation and their interactions with leukemia cells. Moreover, these determinations are all real-time, more facile than traditional complicated methods, and more of a true representation of what is happening in vivo as the measurements are done with the whole cell system rather than by analyzing a single biomarker.

Based on our previous findings, we believe that EC activation and subsequent leukemia cell adherence can serve as a novel model, and first ever example, to test our strategy. A cross-validation of the QCM results with electrochemical measurements and microscopic observations, in conjunction with our detailed biological study of these events (Pezeshkian et al., 2013), shows a strong coherence of the results. Moreover, the technological and procedural ease of this presented protocol is quite significant. Thus, a simple but efficient strategy is presented here which not only provides important information regarding EC activation and subsequent adherence of leukemia cells in real-time, but also can be employed for discrimination and quantification of two kinds of leukemia cells. Thereby, this biosensor construct aims to identify disease stage and potential for relapse as well as aiding in identifying best course of chemotherapy treatment for patients with leukemia in the near future.

2. Physical origin of the response mechanism

As per many physical models, individual cells can be considered as viscoelastic shells around a liquid core (Wottawah et al., 2005). When they adhere to the QCM surface, they reconfigure to a flat interface depending upon the mechanical strength and the contact force (Honda et al., 2004). This results in a change in mechanical energy of the cell (Katira et al., 2012) which has been described in detail in SI. Briefly, this change is dependent upon three factors: 1) the stretching force on the cells, 2) the number of adherent cells and the type of binding, and 3) the changes in osmotic pressure due to intake and release of materials. Furthermore, due to peculiar response mechanism of the QCM depending upon the generation of shear transverse waves that decay rapidly within a viscous media such as living cells, the mechanical properties of the narrow cleft between cell and substrate can only influence the composite QCM response (Wegener et al., 2000) while the apical cell surface having no impact as depicted in Fig. 1. This response can be analyzed by Mason′s model (Bandey et al., 1999) connecting energy loss experienced by an acoustic wave during its propagation to the mass and viscoelastic changes in the contacting medium. For interpreting our system comprising ECs and adherent leukemia cells, the model of overall mechanical energy can be utilized as discussed in SI. It is well known that the activation of ECs by the external or internal stimuli or by activating agents like TNF-α results in a morphological change in EC and reduced cell-surface contact to the QCM substrate. This should result in a reduction of stored energy affecting the inductance as well as frequency. The frequency of the QCM electrode should increase because of higher oscillation due to lower load. On the other hand, due to decreased cell contact to the substrate, the cell bodies lie at larger distances from the oscillating QCM electrode. Thus, the decay length of the penetrating waves will be higher resulting into a decreased damping of the waves, and decreased motional resistance (R_1). Both these parameters are easy to measure and can be quantitatively related to the extent of activation of the ECs. When leukemia cells adhere to activated ECs, a reverse of the above process is initiated. The adherence of leukemia cells can exert a mechanical force, which can be considered as an additional load thereby affecting the QCM frequency. Although this additional load lies outside the range of the penetrating wave as shown in Fig. 1, thus seems to be undetectable. However, the effect of this load can deform the underlying activated ECs. This may change