Detection of cell-material interactions and beyond – A QCM-D study of yeast, bacteria and human cells.

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Understanding and controlling pure cell-material interactions have become increasingly interesting due to the dramatic advancements in the fields of medical implants[1], tissue engineering[2] and cell-based therapies[3]. Current techniques for studying material-cell interactions, are either invasive and depend on end-point detection or falls short of simultaneously monitoring the accompanying internal cellular activities. Employing Quartz crystal microbalance with dissipation monitoring (QCM-D) closes this gap. However, currents QCM-D studies are limited to mammalian cells in complex culture media under static conditions and pre-defined physico-chemical properties, such as temperature, ionic strength and surface hydrophobicity[2]. Therefore, such results may not be generalizable to other cell types and other conditions. In addition, when performed in culture media, studies may yield misleading results in terms of pure substrate-cell interactions due to the contribution of medium to the measured responses. In this study, we employ QCM-D to study the dynamics of cell adhesion under flow and non-flow conditions by monitoring the frequency shift (Δf) of a resonating quartz crystal and the energy dissipation factor (ΔD) at the overlaying layer resulting from different phases of cell adhesion. Most importantly, we provide more insight on the time evolution of different cell-surface adhesion events and the cytoskeletal changes that accompany these phases by analysing the time-dependent cell viscoelastic index ($\Delta D/\Delta f$). Finally, we evaluate the effect of physico-chemical properties, including medium temperature, ionic strength and surface wetting on the different cell adhesion phases.

Yeast cells (*Saccharomyces cerevisiae*) were used as a model for eukaryotic cells and the adhesion profiles compared with that of a typical human cell; the human embryonic kidney (HEK) cell, while E. coli was used as a model for bacterial. Atomic force microscopy and 3D optical microscopy were used for morphological analysis of cells on substrates. Contact Angle (CA) measurements were performed on all surfaces for surface wetting analysis, while cell viability analysis was performed on samples after measurements to assess the viability of the cells under different experimental conditions.

The results show that short term cell adsorption under flow follows a non-linear behaviour and depends on the cell type. In addition, under static conditions, the adhesion of yeast cells involves a series of phases relating to their internal organization and cell surface contact area. As shown in Figure 1, these include an initial lag phase (phase I) attributed to rounded and loosely attached cells. During this phase, the viscoelastic index of the cells ($\Delta D/\Delta f$) depicted in the insert increases sharply, thus indicating that the cells undergo major internal re-organization and become more rigid. Such internal changes can be attributed to the production of adhesion proteins required for strong cell anchoring and spreading. In the next phase, ΔD and Δf change sharply to their corresponding plateaus as the cells achieve maximum spreading and consequently maximum adhesion. $\Delta D/\Delta f$ however become fairly constant until the later part of this phase, suggesting that the measured response is mostly due to cell spreading. In the final

phase (III), the trends in ΔD and Δf are reversed in a fashion that is indicative of the reduction in cell contact area, as reflected in the fairly constant ΔD/Δf until about the 20th hour, probably due to the disassembly of adhesion bonds. More interestingly, we show that these adhesion events are strongly modulated by temperature, ionic strength and the wetting properties of the substrate surface.

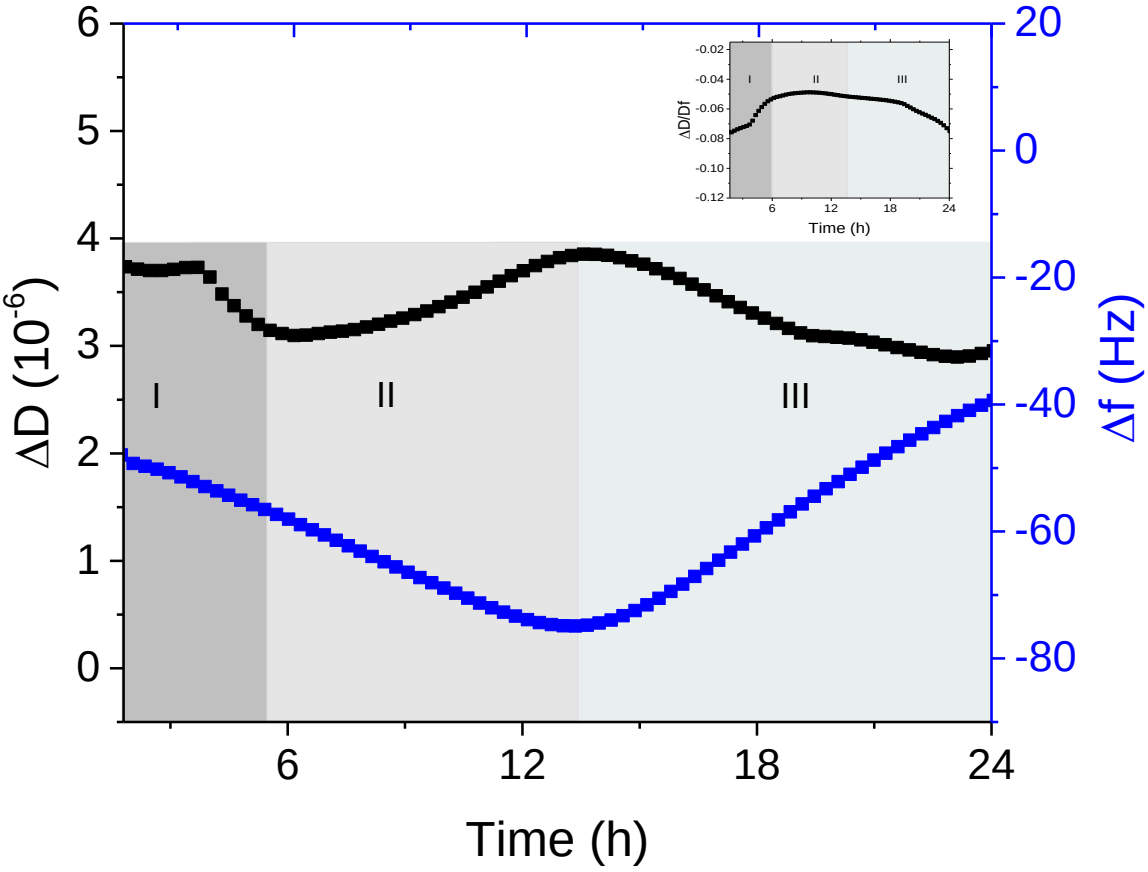

Figure 1: Yeast cell adhesion on silica under non-flow conditions in phosphate buffered saline depicting cytoskeletal changes that accompany cell spreading and desorption.

In conclusion, this study has shown that eukaryotic cells undergo various phases during their interaction with substrates, including a lag phase, spreading phase and a detachment phase. In addition, these phases are strongly modulated by the temperature, ionic strength, and surface wetting properties. The adhesion profiles of bacteria are markedly different, suggesting that the underlying mechanisms for bacteria adhesion may be different from those of eukaryotic cells. However, we show that bacteria cell adhesion is equally strongly controlled by physico-chemical factors.

References

1. M. E. Stamp, A. M. Jötten, P. W. Kudella, D. Breyer, F. G. Strobl, T. M. Geislinger, C. Westerhausen, "Exploring the Limits of Cell Adhesion under Shear Stress within Physiological Conditions and beyond on a Chip". *Diagnostics*, vol. *6*(4), pp. 38, 2016.
2. E. Watarai, R. Matsuno, T. Konno, K. Ishihara, M. Takai, "QCM-D analysis of material–cell interactions targeting a single cell during initial cell attachment". Sensors and Actuators B: Chemical, vol. 171 pp. 1297-1302, 2012
3. Y. K. Wang, C. S. Chen, "Cell adhesion and mechanical stimulation in the regulation of mesenchymal stem cell differentiation". *Journal of cellular and molecular medicine*, vol. *17*(7), pp. 823-832, 2013.