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QCM-D technology as a new platform for real-time monitoring in biology, medicine and engineering

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QCM-D technology is a surface-sensitive technique for real-time monitoring and characterization of (bio)-layers on a surface with regard to adsorption and desorption events, molecular interactions and structural properties. To date, this old-established technique is becoming increasingly important for study of cellular processes both, in nanotechnology and cell biology [1-4]. Interactions between molecules, cells or cells and their immediate environment are observable at the surface of sensor in the natural unaltered condition. QCM-D thus provides a unique insight into the complex world of biology.

The measuring principle

The sensor which is composed of a quartz oscillator is at the heart of the QCM-D technology. This quartz is excited by an alternating electric voltage to mechanical vibrations. By the deposition of very small particles, proteins, bacteria, viruses or cells on the surface of the sensor this vibration is changed in the resonance frequency and/or amplitude and accordingly in the dissipation. This allows the addition of a billionth of a gram, as well as minute changes in the material properties of deposited layers to be registered. The applications of QCM-D range from the characterization of polymer layers to medical issues including detection of pathogens, autoimmune diseases, protein interactions, cell cultures and the coagulation status. In a special way the dynamics of cellular networks or synthesis of macromolecular 3D structures is displayed. The decisive parameters, i) the frequency as probe of the mass and if applicable the coating layer's thickness [5] and ii) the dissipation as a probe of the stiffness / viscoelasticity [6] are detected simultaneously (Fig. 1).

The changes in these parameters closely correlate with binding or interaction events as well as with changes in the structural properties of the surface layer. In Fig. 1, the changes in frequency and dissipation (respectively attenuation) are schematically shown for three typical cases. Binding and arrangement of small globular molecules to rigid homogeneous layers leads (because of the change in mass) to a change in frequency, with an un-changed dissipation (constant viscoelasticity, Fig. 1A). A change in the viscosity of pure (Newtonian) liquids, for example, water compared with glycerin, results in a characteristically decreasing frequency while the same amount is increasing in the damping (Fig. 1B).

The addition of large ‚soft' molecules, such as immune cells or bacterial cells in biofilms also leads to a frequency and dissipation shift in opposite directions, as yet, with different amplitudes (Fig. 1C). The separate recording of frequency and dissipation allows the detailed analysis of processes at the sensor surface. In particular, the additional detection of the dissipation parameter significantly contributes to the deciphering of trans-formations in ‚soft' cellular layers [7-9] (Fig. 1C).

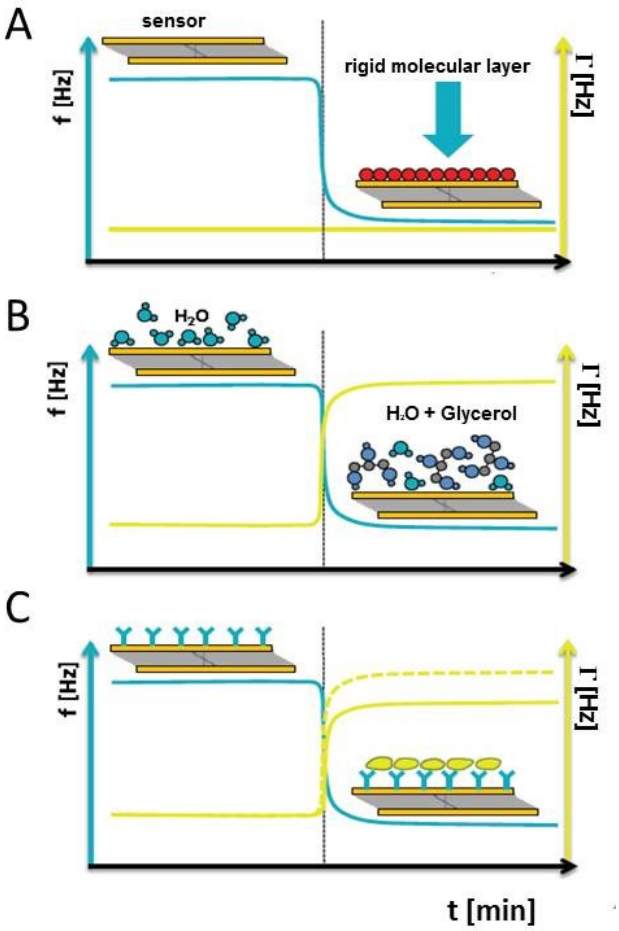

Fig. 1: QCM - D technology on biological samples. Idealized signal progression of the two most critical QCM-D parameters: resonance frequency, f, (blue line) and damping, Γ (respectively dissipation) (yellow line). The relationship between damping and dissipation, D, is given by D = 2Γ/fr (see www.3t-analytik.de/technologies/qcm-d/what-qcm-d). Sensor response during the formation of a rigid layer of small globular molecules (red circles) (A), a change in viscosity of a pure Newtonian solution (B) and formation of a viscoelastic layer from bacteria or immune cells (yellow ovals) (C).

Biofilm morphology and dynamics: adhesion, growth and removal monitored in real time

It is well established that bacteria form complex intercellular communication networks [10]. This capability supports the coexistence of microorganism consortia (bacteria, fungi, yeasts, protozoa, etc.). The integration of these cells in a matrix of extracellular polymeric substances (EPS) results in the formation of so-called biofilms [10].

As a form of survival mechanism, biofilms allow bacteria to adapt to a wide-range of environmental stimuli, making them highly

resistant to extreme conditions and antimicrobial agents. In the human body, biofilms are a frequent precursor to infections [11]. Importantly, biofilms are associated with a high proportion of nosocomial infections with potentially fatal outcome. Thus, biofilms have become central focus of latest research studies. In this study, the biofilm - forming *Pseudomonas aeruginosa (P. aeruginosa)* which is encountered with prevalence in hospitals, is chosen as target organism. Due to the high percentage of antibiotic resistance found in this species, treatment is faced with great difficulty [12]. A portfolio of effective measures to contain the medically significant biofilms is much-needed. For this purpose, a basic understanding of the bacterial cell morphology is required. The development of new platforms for investigating bacterial growth and removal from surfaces is critical for preventing human infection. QCM-D is an excellent approach for these studies, due to its ability to monitor small mass, and viscosity changes in a highly sensitive, localized environment. The morphology of a biofilm contains key information about the function, community diversity, assembly and disassembly, as well as the resistance to disinfectants. It has been demonstrated that by QCM-D, the smallest mass changes (> 5 ng) associated with the bacterial colonization, but also changes in the physical state of the cellular layers during biofilm maturation can be detected in real-time [13-15].

The formation of the bacterial biofilm and the effectivity of surface-active agents in removal of this biofilm have been monitored in real time by QCM-D (Fig. 2, [16]). The changes in frequencies and dissipation reflect the different stages of bacteria colonization and biofilm development at the interface between the sensor and bacterial cells. The initially observed transient sharp increase in dissipation with minor change in the frequency suggests that bacteria have attached in a highly flexible state likely with their poles and/or appendices (Fig. 2B; phase I). This supports the current concept of bacterial adhesion to surfaces by the so-called surface walk [17, 18]. The adhesion phase leads into a phase of constant increase of stably attached bacteria which layer the sensor in an increasingly viscoelastic manner (Fig. 2B; phase II). This indicates the progressive development of bacterial density and the extracellular polymer substances (EPS), and thus maturation of the biofilm. Hitherto, few experimental studies of the formation and benefits of specific physical structures in mixed microbial communities have been conducted. The adhesion of *P. aeruginosa*, the formation of viscoelastic layers through colonization and biofilm formation are monitored by mass and viscosity changes. The frequency and damping displacements closely reflect the increase in the number of attached bacteria and the viscoelastic properties respectively during maturation of biofilm and cell embedding in the EPS matrix (Fig. 2B, Phase II). Particularly, the dissipation behavior addresses the role of a specific physical structure in biofilm initiation and persistence.

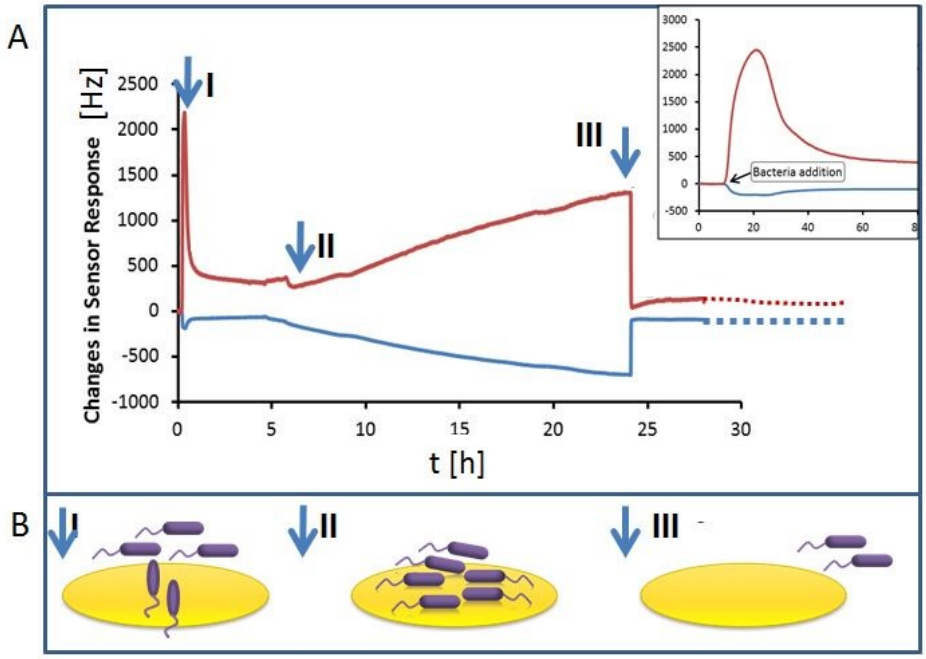

Fig. 2: Bacterial adhesion, growth and removal monitored by QCM-D-technology. (A) Frequency (blue) und damping signal (red) progression during addition of *P. aeruginosa* (I), growth/biofilm-formation (II) and biofilm-removal (III). The Inset shows the process of adhesion during the first 60 min after bacteria inlet (black arrow; t=60 min). Arrows mark the addition of bacteria, of growth medium and detergent (SDS). (B) Model scheme of bacteria dynamics at the sensor surface: I) bacteria addition and adhesion, II) addition of medium, growth of the bacterial layer and increase in the hydro-viscous state due to emergence of the EPS; III) detergent addition: removal of bacterial film from the sensor [16].

Finally, the effectiveness of bacteria removal by biocides, for example, detergents is instantly indicated by the frequency and damping signals (Fig. 2B, phase III). Thus, QCM-D is providing an optimal screening tool for suitable surface active agents with the aim to develop future strategies to efficiently repel bacterial infections. QCM-D technology is a novel platform to address these issues due to its unique ability to monitor nanoscale mass and structural changes of living cellular layers in a highly sensitive and real time manner.

Tissue Engineering: functionalization of biomimetic nanomaterials

Biomimetic cell matrix constructs modeled based on the intricate nanofibrous architecture of the natural extracellular matrix (ECM) have already made remarkable achievements in the reconstruction of tissues in animal models via tissue engineering. A constant tissue repair requires ideally matrix constructs with bioactive molecules, including growth factors such as bone morphogenetic proteins. These factors are equipped to accelerate the necessary specific ECM production and to promote tissue integration. Great progress can be expected from the combination of the latest achievements in the construction of nano-engineered ECM analogs and the enrichment of the surface of these nanomaterials with bioactive compounds [19, 20]. QCM-D has been successfully employed as a real-time monitor of coating of nanofibrous poly-ε-Caprolacton (PCL)-coated nanoparticles with functional bio-compounds [21]. The resonant frequency shifts reflect the gradual coating of the PCL-nanofiber scaffold with the linker amino acids, p-lysine and p-glutamate. At the final coating stage, the replenishment of the nanostructures' cisterns with fibroblasts FGF2 growth factors for bio-activation is monitored (Fig. 3) [21, 22].

Conclusions

Owing to its high versatility and flexibility, the QCM-D technology, is emerging as a highly topical and extremely interesting analytical real time method in biology, nanotechnology etc. It offers superior sensitivity combined with high reliability for real-time detection of e.g. binding reactions, changes in structure and morphology in cellular samples in their natural states. Moreover, QCM-D to date has been applied in a wide range of applications

in synthetic functional materials including polyelectrolytes or nanomaterials.

The outstanding patented 3T analytik sensor design ensures easy handling of the quartz crystal sensor under stress-free conditions. With a unique portfolio of different quartz coatings, such as, elemental metals (Ti, Ag, Cu, Pt, etc.), oxides (SiO_2, Al_2O_3, etc.), hydroxyapatites or polymers, 3T qCell instruments are optimally equipped to satisfy any of your specific requirements. By the straightforward powerful software enabling fully automatic machine control, signal acquisition and processing, you easily manage the measurements and get quick answers. The numerous advantages and unmatched optimization of 3T equipment have advanced the QCM-D technology to hold an increasingly important role among the analytical methods for investigations of molecular interactions and surface phenomena both in biology and technology. Applications range from environmental diagnostics, over fundamental questions of cell-cell interactions, to pharmaceutical development and medical diagnosis and prognosis.

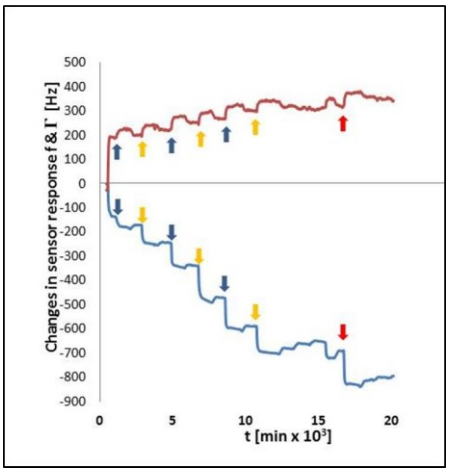

Fig. 3: QCM-D as monitor of the bio-activation of synthetic nanofibers towards biomimetic bone cell-matrix constructs. Frequency (blue) and damping (red) signal shifting during the stepwise coating of the nanofiber scaffold with poly-amino acids , p-lysine (blue arrows), p-glutamic acid (yellow arrows) and the final bio-activation by addition of fibroblast growth factor, FGF2 (red arrow) [22].

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