High-Frequency Multiplex Acoustic Biosensing of TRM Cells and Apoptotic Markers in a Colorectal Cancer Liver Metastasis-on-Chip Model

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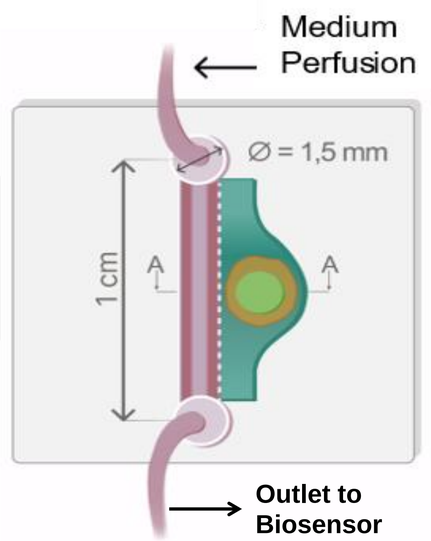
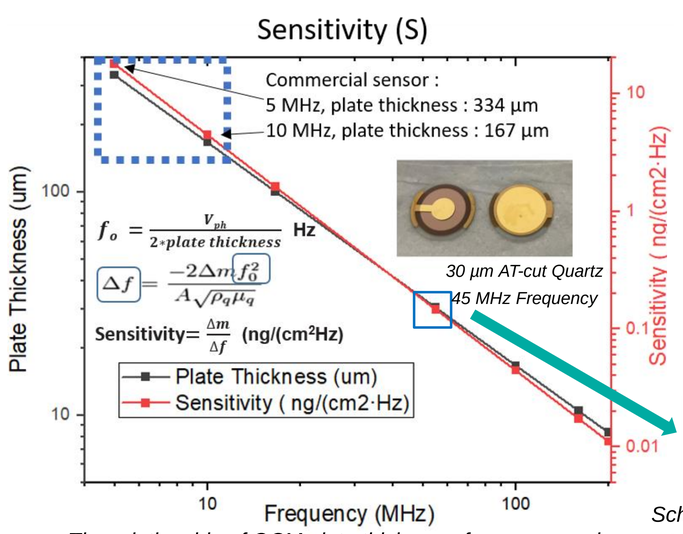
Introduction

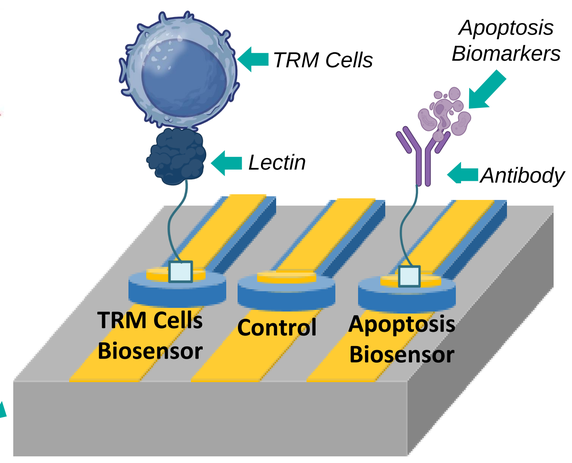
Conventional in vitro and in vivo models remain insufficient for developing effective therapies for colorectal cancer liver metastasis, a leading cause of cancer-related mortality. To address this limitation, we are developing a Microphysiological System (MPS), or Cancer-on-Chip (CoC), that recapitulates the tumor microenvironment to evaluate emerging immunotherapies such as tissue-resident memory T (TRM) cell therapy.
As a planned advancement, we propose the integration of **a multiplexed Quartz-on-Silicon Microbalance (QSiM) biosensor** for real-time, label-free monitoring. The multiplex QSiM chip is designed to **simultaneously detect TRM cells, apoptosis-related biomarkers, and on-chip controls** within the same system. By enabling higher operating frequencies with improved mechanical stability compared to conventional quartz crystal microbalances (QCM), QSiM is expected **to enhance sensitivity and limit of detection by up to two orders of magnitude.**



High-Frequency & Multiplex QSiM Biosensor

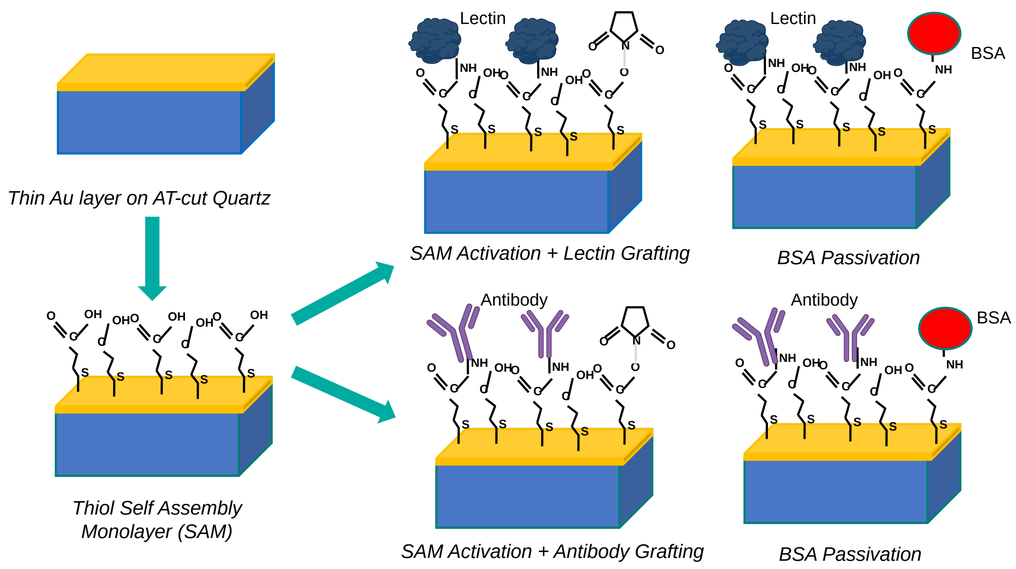


The relationship of QCM plate thickness, frequency and theoretical sensitivity. Higher frequencies and sensitivities are achieved by thinner plate thicknesses.



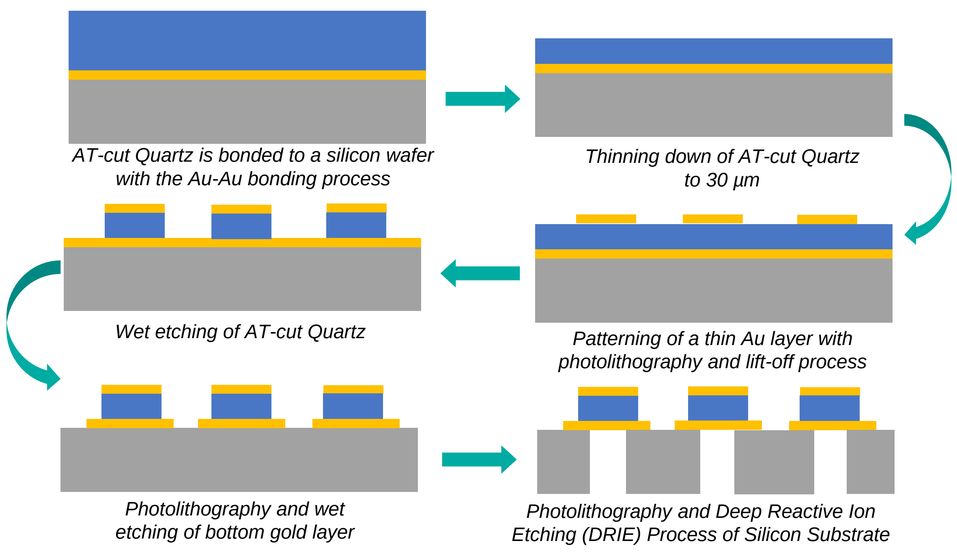
Schematic of the QSiM chip integrates three biosensors: a TRM cell biosensor functionalized with lectin ligands, an apoptosis biomarker biosensor functionalized with antibodies, and a control sensor for baseline and nonspecific signal correction.

Surface Biofunctionalisation

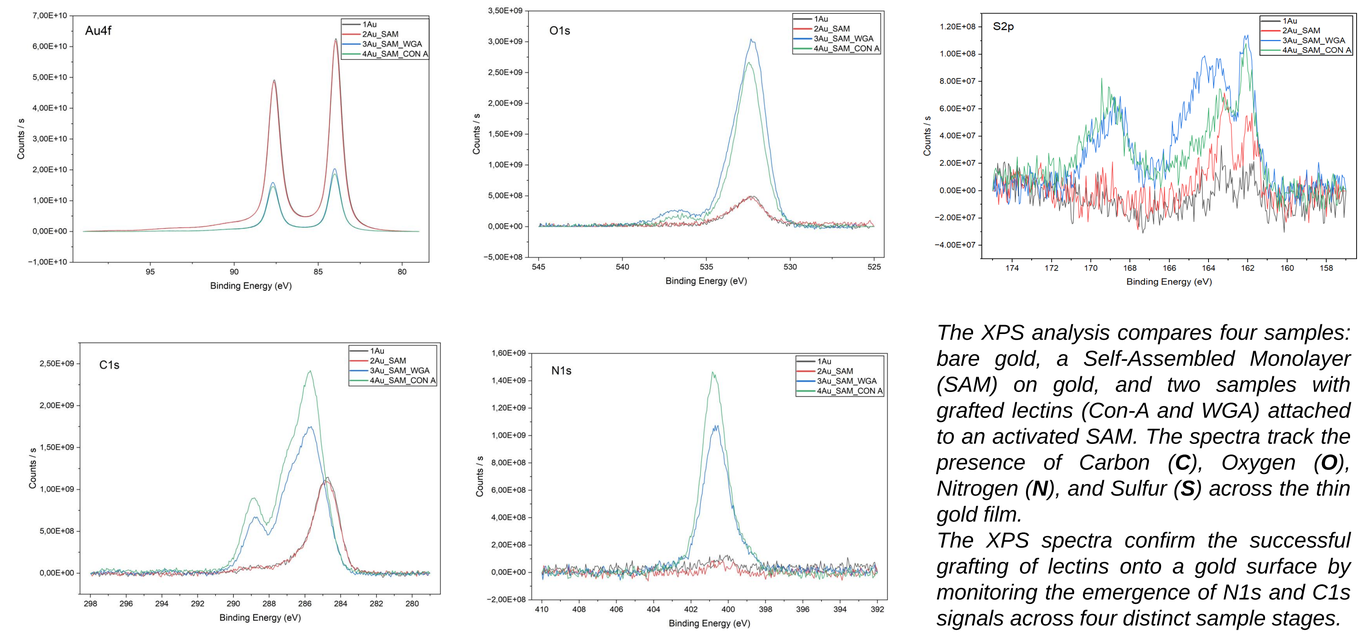


A thin gold layer on AT-cut quartz is functionalized with a thiol self-assembled monolayer (SAM), chemically activated, and grafted with either lectin or antibody ligands. BSA passivation is applied to block nonspecific binding and ensure selective biosensor responses.

Microfabrication Process Flow



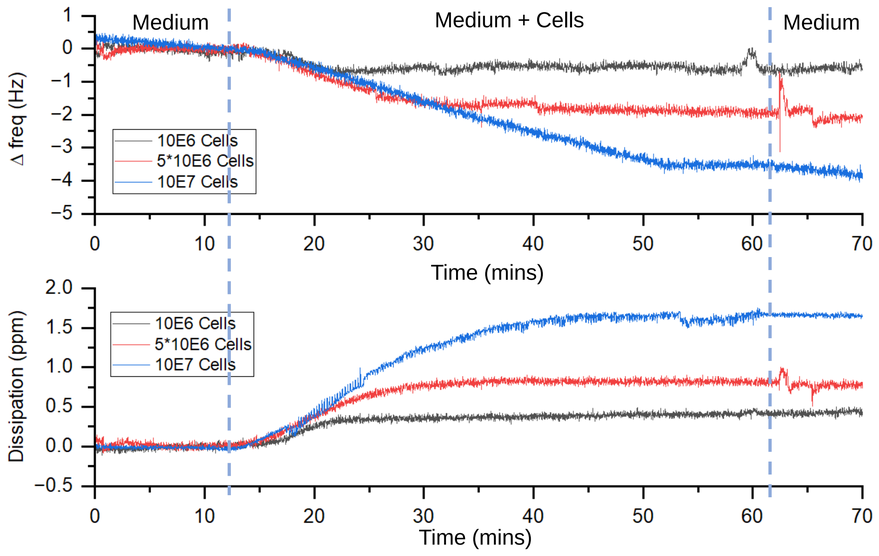
Biointerfaces Characterisation: XPS



*The XPS analysis compares four samples: bare gold, a Self-Assembled Monolayer (SAM) on gold, and two samples with grafted lectins (Con-A and WGA) attached to an activated SAM. The spectra track the presence of Carbon (**C**), Oxygen (**O**), Nitrogen (**N**), and Sulfur (**S**) across the thin gold film.*
The XPS spectra confirm the successful grafting of lectins onto a gold surface by monitoring the emergence of N1s and C1s signals across four distinct sample stages.

Preliminary Result

(Immobilisation and Detection of T-Cell with 5 MHz QCM)



Real-time detection of T-cells using a 5 MHz QCM sensor functionalized with WGA lectin shows a clear concentration-dependent response between 1x10⁶, 5x10⁶ and 1x10⁷ cells/mL.

Conclusion and Perspective

We have proposed a multiplexed **Quartz-on-Silicon Microbalance (QSiM)** biosensor platform designed to maximise sensitivity while enabling the **simultaneous detection of multiple biomolecules and on-chip controls**. To evaluate TRM cell therapy within the Cancer-on-Chip system, we developed two distinct functionalization strategies: **lectin grafting for TRM cell capture** and **antibody immobilisation for apoptosis biomarkers**. Successful lectin grafting was confirmed via XPS analysis, showing the characteristic emergence of N1s and C1s signals. Preliminary results using a commercial 5 MHz QCM achieved a Limit of Detection (LoD) of 1x10^6 cells/mL; however, transitioning to the high-frequency **QSiM sensor is expected to improve this LoD to 1x10^4 cells/mL**. Development of the antibody-based apoptosis detection, the microfabrication and final integration of the multiplexed QSiM biosensor are currently ongoing.

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