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Microphysiological analytical chip with non-invasive Raman microspectroscopy analysis of epithelial permeability and inflammatory biomarkers

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Summary:

The presented work reports the non-invasive detection of multiple biomarkers in a custom microphysiological chip, designed for co-culture tissues and organ-on-chip investigations. The proposed SERS-on-chip detection system is optimized for the specific detection of two colorectal cancer biomarkers at physiological concentrations, the Oncostatin M and TNF-α. Under optimized pH conditions, tuned on-board, the ML algorithm in F12K medium achieved 99 % accuracy for Oncostatin M detection and 92 % accuracy vs TNF-α detection.

Keywords: Microphysiological chip, biomarkers chemical sensing, lab-on-chip, organ-on-chip, Raman sensing

Background, Motivation an Objective

The need for a rapid, specific and sensitive diagnostic system to identify unique antigens biomarkers, such as those associated with inflammation or cancer is crucial in modern biomedical research. The detection of these markers in complex matrices like body fluids or cell culture media is particularly challenging due to their low concentration, as well as the presence of multiple interfering components. SERS-enhanced detection combines high sensitivity and potential specificity with the added advantages of water non-interference and compatibility with transparent polymeric materials, making it a perfect fit for organ-on-chip non-invasive diagnostic methods[1-3].

Description of the New Method or System

The presented work reports the non-invasive detection of multiple biomarkers in a custom microphysiological platform, designed for co-culture tissues. However, one of the main limitations of SERS method lies in spectral overlap, which results from the binding of multiple analytes to the substrate. The proposed algorithm approaches this limitation by functionalizing the surface of a miniature SERS substrate integrated in a measurement chamber of the chip with a self-assembled monolayer (SAM) of 3-mercaptopropionic acid (3-MPA). Due to the pH modification of the cell culture medium vs the target protein isoelectric points, the carboxylic groups of 3-MPA can interact in a more selective way with a reduced number of molecules (target/acid protonation status modification), enhancing the Machine Learning (ML) classification performance. The proposed SERS-on-chip detection system was optimized for the specific detection of two colorectal cancer biomarkers at physiological concentrations, the Oncostatin M and TNF-α.

Results

The pH modulation is managed at chip level with an embedded microfluidic mixer, without affecting the cell culture chamber and acquiring the Raman spectra through the chip lid (Fig. 1). The obtained Raman spectra (500-2000 cm^{-1}, 632.8 nm laser, 1800 gr/mm grating, 20x objective) collected on a microfluidic chip onto a 37°C stage were analyzed using a supervised Machine Learning approach and then correlated with standard TEER measurements of epithelial permeability in order to classify the different junctions damages (T0, T2h, T4h of exposure the

chelating agent) in a non-destructive way. This approach allows to detect TJ status at different agent exposure time with a validation accuracy of 82% and a test accuracy of 96%. Raman microspectroscopy, combined with support vector machines algorithm, represents a promising method for improving the diagnostic precision of microphysiological platforms. Furthermore, this tool represents a non-destructive and promising method for disease prediction, physiological parameters monitoring and drugs PK/PD screening. Under optimized pH conditions, the ML algorithm in F12K medium achieved 99 % accuracy for Oncostatin M detection and 92 % accuracy vs TNF-α detection (Fig. 2). A complementary proposed study was focused to analyse with same setup the structural and biochemical changes in a Caco-2 intestinal epithelial model before and after treatment with a calcium chelating agent. Two different data-splitting approaches, “sample-based” and “spectra-based,” were also compared. Raman microspectroscopy results were confirmed by TEER measurements and immunofluorescence staining.

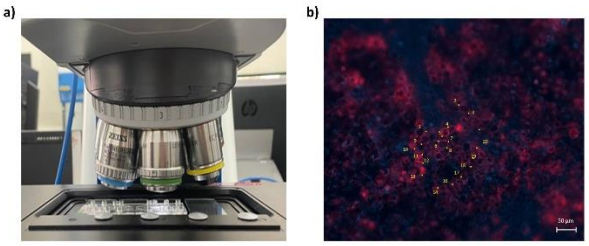

Fig.1 a) MicroRaman Microscope with motorized stage for 2D cell culture mapping; b) Immunofluorescent staining of Caco-2 cells intestinal epithelium, marked with 20 points from inter-cellular junctions analyzed with Raman spectroscopy (Red e-cadherins on tight junctions, Blue nuclei).

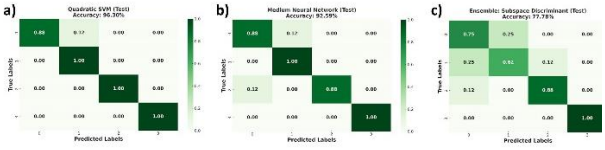

Fig.2 Heatmap of the test accuracy across four classes, corresponding to different EGTA exposure times (T0, T2h, T4h and empty membrane) of Quadratic SVM (A), Medium Neural Network (B) and Ensemble (C) algorithms.

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