Development of a microfluidic lab-on-a-chip platform for breast cancer early diagnosis

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MicroBioMed is a selected project in the Operational Program INTERREG IV-A Euregio-Meuse-Rhine. The goal is to develop a network of expertise through the development of a lab-on-chip demonstrator for the immunological detection of breast cancer (in vitro diagnostics).

Target antigens

Potential protein serum biomarkers were identified and subsequently validated by three different steps: mRNA expression was determined in benign and malignant breast cancer cell lines.

Next, expression of candidate genes was measured on mRNA and protein level using a collection of fresh frozen breast cancer specimens and healthy breast tissues. For candidates confirmed as upregulated in breast cancer tissue, Western Blot analysis and ELISA assays were performed using human serum samples.

The performance of a multi-marker-panel was optimised using receiver operator characteristics (ROC) curve analysis.

Antibodies generation

Different soluble and membrane-bound breast-cancer related antigens (BCRAs) were used to immunise BALB/c mice. After sufficient titers were reached, spleens were removed and used for standard hybridoma technology.

After several rounds of screenings, monoclonal antibodies (mAbs) were identified and produced. The different mAbs were tested for their binding to the corresponding BCRAs and ELISA-based assays were evaluated.

MUC1-based reference assay

MUC1-1 (MUC1) is a well-known and highly validated tumor specific antigen which has been chosen to evaluate the potential of the different new designs.

In parallel, with the use of flow cytometry, an assay has been designed which combines two antibodies against MUC1 (214D4, unspecific, and 5E5, specific to cancer Mucin) in order to generate a more sensitive and specific detection of cancer cells.

T47D breast cancer cells were still detectable at 1 in 1x10^6 of PBMCs from a healthy donor.

Surface modification: micropillars

The silicon micropillar array works as an autonomous capillary pump, capable of transporting a specific amount of bio-samples into the sensing area without external pumping requirement.

Surface plasmon resonance

Gold nanoparticles (AuNPs) on a silicon array are being used for a label-free biosensing. Tests were performed with anti-MUC1 antibody (214D4):

Successful Anti-MUC1 antibody coupling on gold surface (monitored by SPR) via mixed “COOH” and “OH” SAM.

Impedance spectroscopy

Impedance change measured at 1.6 kHz (covalent binding in red and non-covalent in blue)

Surface acoustic wave

A surface acoustic wave chip was produced using a piezoelectric substrate and lithographically structured gold electrodes.

When a probe (e.g. proteins) is being deposited, the change in the frequency of the acoustic waves can be measured.

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