MicroBioMed

Microtechnologies for Biomedicine Applications

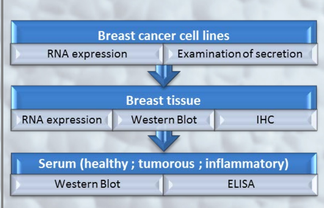
Research on lab-on-a-chip for *in-vitro* diagnostics

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

Karl Fleury-Frenette (University of Liège, Centre Spatial de Liège, Belgium), Christiane Puettmann and Christoph Stein (RWTH Aachen University, Helmholtz Institute for Biomedical Engineering, Dept. of Experimental Medicine and Immunotherapy, Aachen, Germany), Liesbet Lagae (IMEC, Leuven, Belgium), Patrick Wagner (Institute for Materials Research IMO, Hasselt University, Diepenbeek, Belgium), Edgar Dahl and Stefan Garzcyk (Molecular Oncology Group, Institute of Pathology, Medical Faculty of the RWTH Aachen University, Aachen, Germany), Gerard Bos and Evelien Bouwmans (Department of Internal Medicine, Division of Haematology, Maastricht University Medical Center, Maastricht, the Netherlands), Uwe Schnakenberg and Andreas Buchenauer (Institute of Materials in Electrical Engineering 1, RWTH Aachen University, Aachen, Germany), Aline Lausberg (University of Liège, GIGA, Liège, Belgium), Joseph Bernier (University of Liège, Centre Spatial de Liège, Belgium)

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).

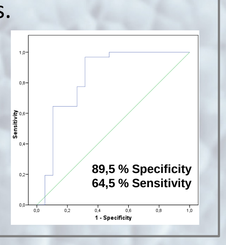
Target antigens



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.

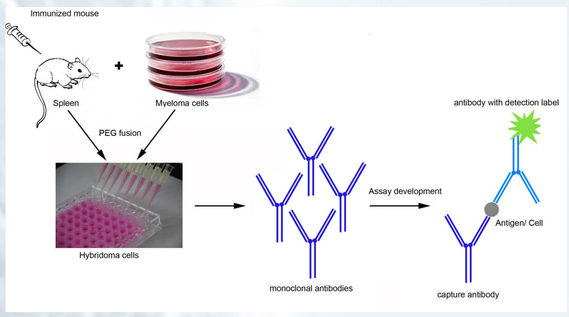
The performance of a multi-marker-panel was optimised using receiver operator characteristics (ROC) curve analysis.



ROC curve analysis for marker-panel ➔

Antibodies generation

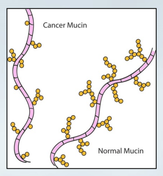
Different soluble and membrane-bound breast-cancer related antigens (BCRA) were used to immunize BALB/c mice. After sufficient titers were reached, spleens were removed and used for standard hybridoma technology.

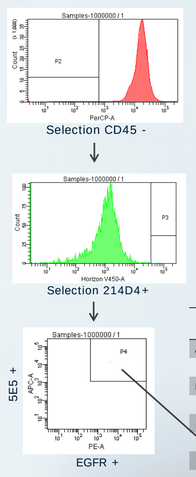


After several rounds of screenings, **monoclonal antibodies (mAb)** were identified and produced. The different mAbs were tested for their binding to the corresponding BCRAs and ELISA-based assays were evaluated.

MUC1-based reference assay

Mucin-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.



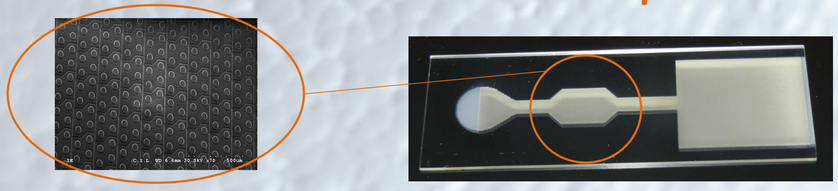


In parallel, with the use of **flow cytometry**, an assay has been designed which combines two antibodies against MUC1 (214D4, unspecific, and 5E5, specific to cancer Mucin) in order to generate a more sensitive and specific detection of cancer cells.

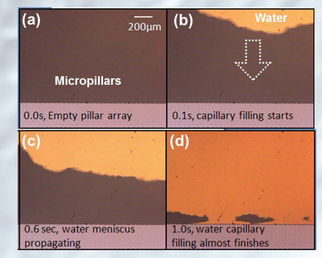
Sample			Final events
Alone		PBMC	0
		T47D	319
Mixed	10.000	1%	53
	1.000	0,1%	12
	100	0,01%	10
	10	0,001%	4
	1	0,0001%	1

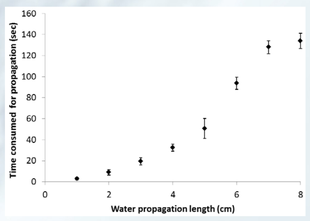
T47D breast cancer cells were still detectable at 1 in $1x10^6$ of PBMCs from a healthy donor

Surface modification : micropillars

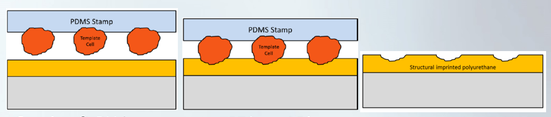


The silicon micropillar array works as an **autonomous capillary pump**, capable of transporting a specific amount of bio-samples into the sensing area without external pumping requirement.



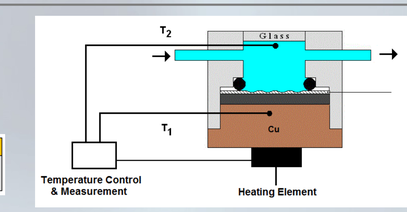


Imprinted polymers

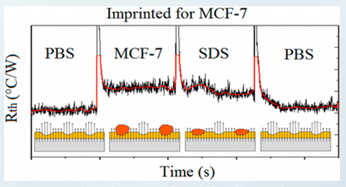


Semi-soft PU layer on Al chip

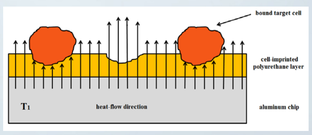
65°c – 12h



Sensor set-up for heat-conduction measurements (T_1 = **37.0°C**)



Binding of cells (PBS) → cell lysis (SDS) → rinsing (PBS)



Bound cells **block** the thermal current: heat-transfer resistance R_{th} increases, **T_2 will drop**

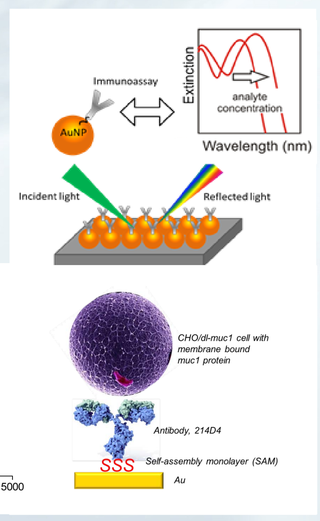
Patent application: WO2012/076349A1

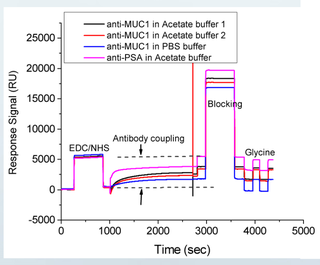
Reference: **Kasper Eersels *et al.*, *ACS Applied Materials and Interfaces* 5, 7258 – 7267 (2013).**

Surface plasmon resonance

Gold nanoparticles (AuNPs) on a silicon array are being used for a label-free biosensing.

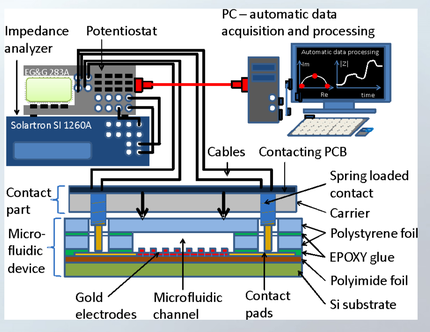
Tests were performed with anti-MUC1 antibody (214D4):

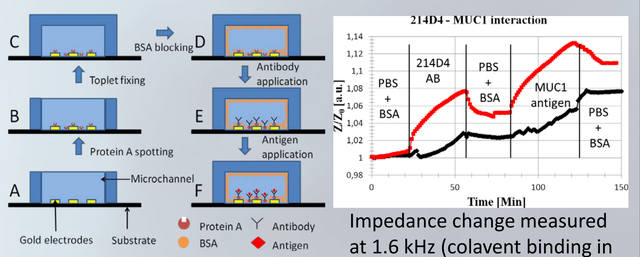




Successful Anti-MUC1 antibody coupling on gold surface (monitored by SPR) via mixed “COOH” and “OH” SAM

Impedance spectroscopy

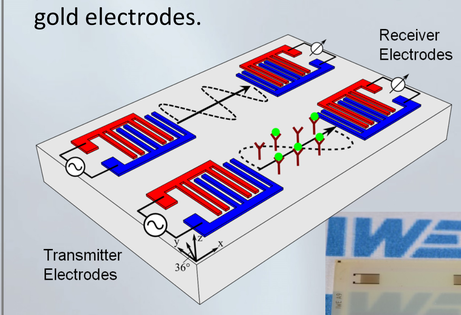


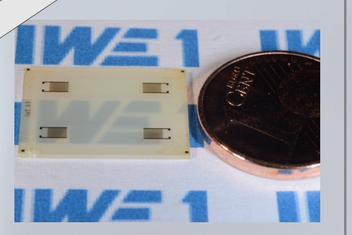


Impedance change measured at 1.6 kHz (colavent binding in red and non-covalent in blue)

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

Selected project in the Operational Program INTERREG IV-A Euregio-Meuse-Rhine

www.microbiomed.eu

With the support of the European Regional Development Fund (ERDF).
The European Commission invests in your future.

PARTNERS

EUREGIO MEUSE-RHIN MAAS-RHEIN · Wallonie · Ministerium für Wirtschaft, Energie, Bauen, Wohnen und Verkehr des Landes Nordrhein-Westfalen · Ministerie van Economische Zaken · provincie Limburg · PROVINCIE VLAAMS • BRABANT