



NANO-IMMUNO ASSAY DEVELOPMENT FOR THE DETECTION OF CANCER BIOMARKERS

Elena Ambrosetti, Alessandro Bosco, Loredana Casalis

Nanoinnovation Lab, Elettra Sincrotrone Trieste S.C.p.A., Strada Statale 14 – km 163,5
Area Science Park, 34149 Basovizza, Trieste

Abstract — The quantitative analysis of protein markers is a promising strategy in diagnosis, prognosis and therapy monitoring of malignant tumors. The optimization of the clinical detection of these markers is directed towards the use of a small volume system.

We developed a multiplexing nano-immuno array for proteomic analysis in low sample volumes with potential capability of pathological screening of cancer biomarkers.

Nanografting, a tip assisted Atomic Force Microscopy (AFM) nanolithography technique is used to fabricate DNA nanoarrays. DNA nanospots created by nanografting are exploited in order to immobilize DNA-antibody conjugates that recognize specific proteins of interest.

The determination of the antigen content of a biological sample was obtained from the analysis of AFM topographic profiles of the nanopatches before and after the incubation.

As a proof of principle, we focused on the biomarker Human Epidermal Growth Factor Receptor 2 (Her2), relevant antigen found in some human cancers such as breast, lung and gastric ones.

By measuring spot height variation we performed the detection of low concentrations of the biomarker and we were able to optimize the device sensitivity by correlating the density of the DNA-antibody conjugates on the surface and their capability to bind the Extra Cellular Domain (ECD) of Her2.

As future perspectives we are also exploring the use of new binders, as aptamers and nanobodies (VHH), as new tool for the recognition of specific biomarkers with higher affinity.

Index Terms — AFM, nano-immuno assay, cancer biomarker

1 BACKGROUND

A promising strategy in clinical practice for cancer prevention is to perform an early non-invasive population screening. The detection and quantitative analysis of “new generation” cancer biomarkers, a new category of biomolecules released in the bloodstream from tumor tissue and hence important in primary tumors and in the metastasis occurrence, can be effectively exploited in cancer diagnosis and prognosis and in the monitoring of the response to therapeutic treatment.

Nowadays all the techniques used for the detection of these cancer biomarkers have some limitations: they often require large sample volumes, work with labeled-molecules and are characterized by multi-step and laborious experimental procedures, which increase analysis cost and time and limit these techniques from point-of-care medical diagnostic applications. One strategy for overcoming these problems and optimizing a non-invasive “liquid biopsy” is to develop nanoarrays, able to do a multiplexing analysis with high sensitivity, in very small quantities of sample and with a label-free technique.



2 OBJECTIVES

The main goal of this work is to set-up an highly sensitive nano-immuno assay capable to do a parallel analysis of different cancer biomarkers in a small sample volume and with a label-free technique.

As a proof of principle, we focused on the Human Epidermal Growth Factor Receptor 2 (Her2), a biomarker specific for breast, lung and gastric cancers.

3 APPROACH & METHODS

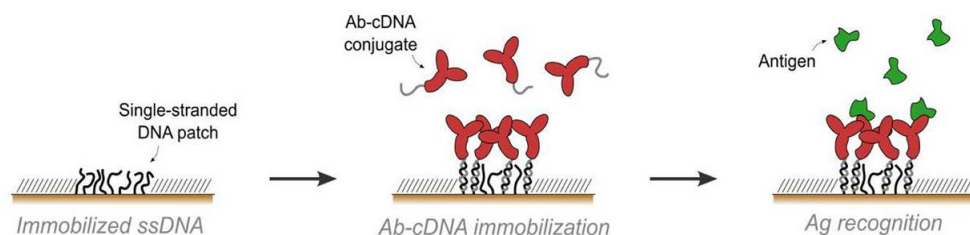
General approach

Atomic Force Microscopy is exploited in order to fabricate DNA nanoarrays; this is the first step towards the immobilization of antibodies specific to certain proteins of interest, through DNA-directed-immobilization (DDI) of DNA-conjugated antibodies.

Methods

Atomic Force Microscopy (AFM): high-resolution scanning probe microscopy that exploits the interactions between a tip and the sample surface to collect its topography at sub-nanometric scale.

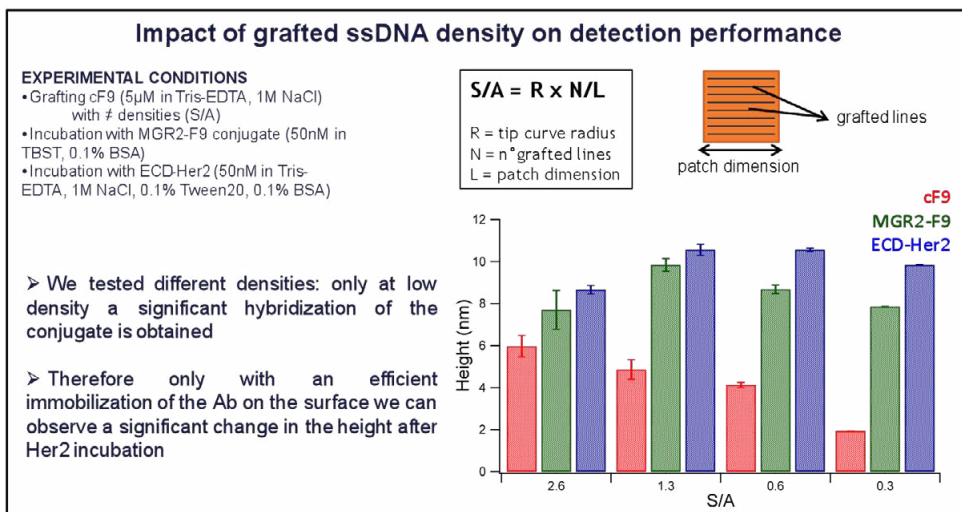
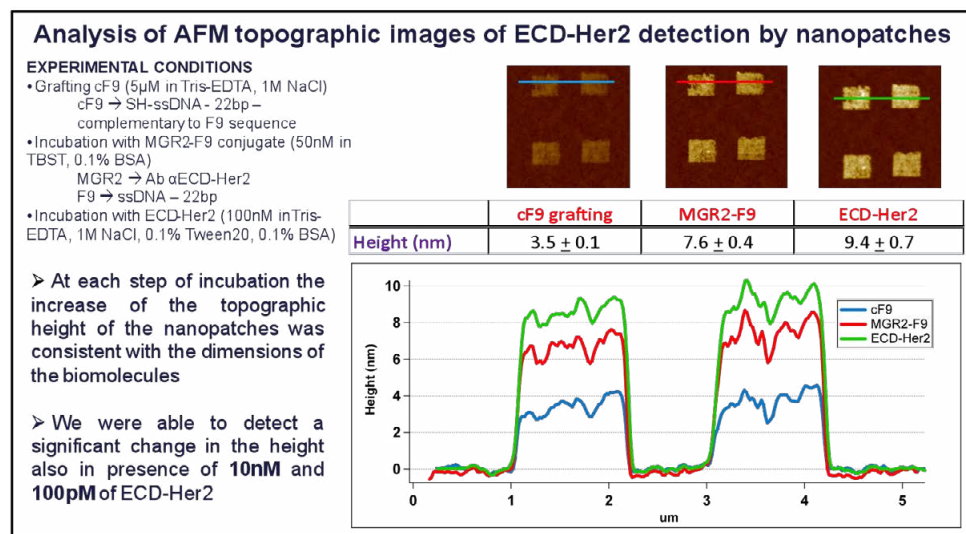
Nanografting: AFM nanofabrication method that combines the displacement of selected thiol molecules of a Self Assembled Monolayer (SAM) adsorbed on a gold surface by new thiol molecules, under high force scanning track of the AFM tip.



DNA-antibodies Conjugation: "click chemistry" method by which first both protein and DNA are functionalized with a chemical group, then these two groups are linked obtaining the conjugate; the final product is quantifiable spectrophotometrically.

Detection system: measure of the topographic height of the nanopatches by AFM with respect to the surrounding thiol carpet. The binding of the molecules at each step of the experiment should determine a change in the height, consistent with the dimension of the molecules.

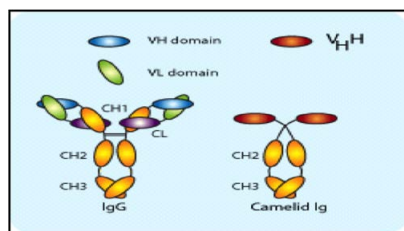
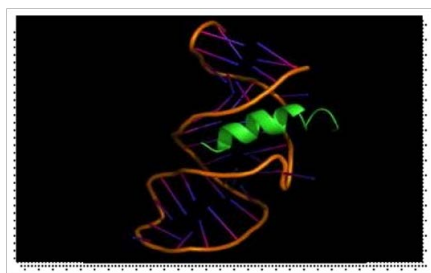
4 RESULTS



5 FUTURE PERSPECTIVES

Perform and optimize multiplexing analysis on tumor cell lysates and then in blood serum samples
Explore the feasibility of other biomolecules that could be exploited for the detection with higher sensitivity:

- APTAMERS: single-stranded oligonucleotides that have been engineered through repeated rounds of in vitro selection (SELEX) to bind to various molecular targets
- VHs: antibody fragments (12-15kDa) consisting of a single monomeric variable antibody domain produced by camelids



6 CURRENT COLLABORATIONS

6.1 With other researchers

- Dr. Elda Tagliabue - Molecular Targeting Unit, Department of Experimental Oncology and Molecular Medicine, Fondazione IRCCS-Istituto Nazionale dei Tumori - Milano, ITALY
- Structural Biology Laboratory - Elettra Sincrotrone Trieste S.C.p.A.
- COBIK - Slovenia

7 CONTACT OR COLLABORATIONS NEEDED

Collaboration with clinical laboratories is needed in order to obtain serum samples of patients with primary or metastatic tumor, undergone or not to therapeutic treatment.

8 COMMUNICATION TOOLS

Email: elena.ambrosetti@elettra.eu

Address: NanoInnovation Lab - Elettra Sincrotrone Trieste S.C.p.A, Strada Statale 14 - km 163,5 in AREA Science Park, 34149 Basovizza, Trieste ITALY