RESULTS 2020

- **Project: New methods of pregnancy monitoring and prenatal diagnosis - MiMoSa**
  COORDINATOR: Nanobiotechnologies Laboratory - INCD for Microtechnology
  Contract no. 67PCCDI / 2018, project director: Dr. Monica Simion

The complex MiMoSa project proposes the development in parallel, through a close interaction, of 4 component projects that aim at obtaining minimally invasive methods that do not endanger the pregnancy, especially in the case of patients at risk, as follows:

- ***early diagnosis*** *of gene mutations using SRY gene detection by surface plasmon technology (project P1);*
- ***assessment of the risk of premature birth*** *by monitoring the level of glucose in saliva (project P2);*
- *analysis of the implications of HPV infections in triggering premature births (project P3);*
- *monitoring contractions in order to evaluate the beginning of early labor (project P4).*

Project 1 - Non-invasive prenatal screening using free circulating fetal DNA extracted from maternal blood

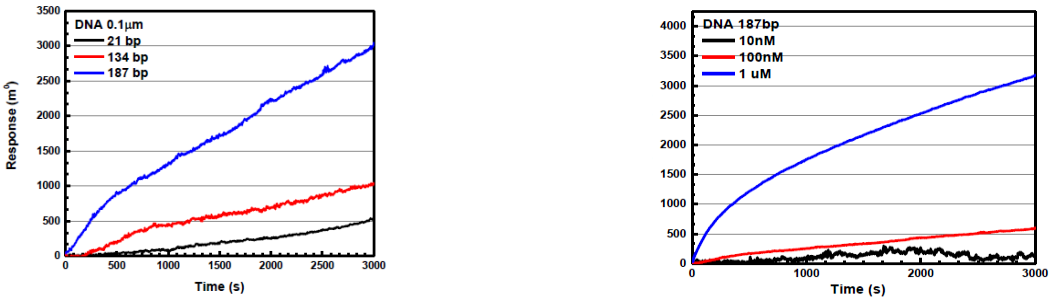
IMT component project director: Dr. Razvan Pascu (Dr. Melania Popescu)

Given the main objective of the project to detect specific DNA sequences obtained by isolating fetal DNA in the mother's blood in the first months of pregnancy, which involves the analysis of picomolar concentrations, finding a method to amplify the SPR signal is necessary.
For this, the amplification of the signal can be obtained by using larger molecules that can be obtained in two ways: (i) the introduction of specific DNA sequences called helpings or (ii) the conjugation of gold nanorods of interest.

(i) ssDNA probes with different lengths and concentrations were immobilized on the Au surface. Through these measurements we investigated the influence of the number of base pairs (bp) on the processes of DNA immobilization and hybridization. The first set of tests aimed at obtaining clear indications related to the dimensions of the molecules that can be detected at the required concentrations of interest.

By attaching single-stranded DNA fragments to the surface of the gold film, we studied the changes in the SPR response for three DNA probes with different length 21, 134 and 187 bp.

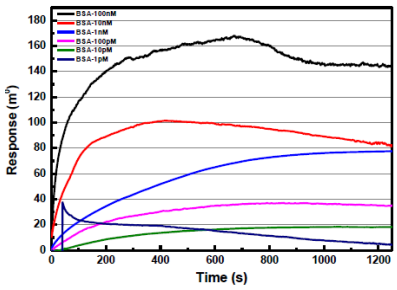
ADN 21 bp probe	6,66 kDa	5' thiol modification 6 carbon spacer
ADN 134 bp probe	41,55 kDa	
ADN 187 bp probe	56,98 kDa	



Sensograms corresponding to DNA immobilization on the surface of the Au sensor

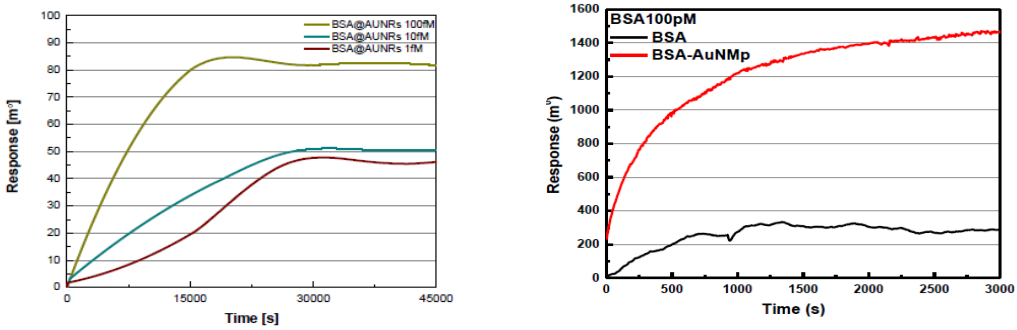
The lowest concentration determined was 10nM for the 187bp size DNA sequence.

To highlight the role of molecular weight on the SPR detection limit, the V fraction of bovine serum albumin (BSA) was used, with MW = 68 kDa. The results confirm that the detection limit can be lowered using high molecular weight molecules. Thus, the most diluted detectable sample had a concentration of 10 pM, for it a maximum response of ~ 150 was recorded, after 20 minutes.



Sensogram corresponding to various concentrations of BSA

(ii) The second method of signal amplification by conjugation with AuNRs was tested for BSA to achieve a greater decrease in the detection limit. The interaction of gold nanorods (AuNRs) with bovine serum albumin protein (BSA) at physiological pH is investigated using as buffer MgCl2 to obtain dilutions of BSA @ AuNRs from a concentration of 1 μM to 1 fM.

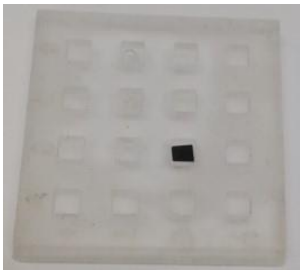


Sensogram corresponding to a) various concentrations of de BSA@AuNRs and b) Signal amplification using AuNRs

The results confirmed that the detection limit of high molecular weight molecules can be reduced by modification with nanoparticles that are responsible for promoting further signal amplification.

Project 3 - Assessment of the risk of premature birth due to HPV infection
IMT component project director: dr. Iuliana Mihalache.

In the design and realization of the test structure were taken into account two main directions: building blades as small as possible so that material consumption is as small as possible, with obvious impact on the production cost of the microarray blade, but also opting for a blade model that it can be introduced into a pre-designed well so that the whole subsequent hybridization process is as efficient as possible.



Thus, a well-type enclosure was created, in which the blade can be placed and the hybridization solution together with the samples to be added on top, in sufficient quantity, later an efficient stirring necessary for a better hybridization can take place. The current dimensions of the silicon blade used are: 3.5mm x 3.5mm, the distance at which each probe was written from the other probes being: 500μm. To make the enclosure, a mold with a 3D printer was designed and made to obtain individual hybridization chambers for each chip tested and to avoid contamination of the samples.

Figure molding with wells in PDMS

For the fluorescent labeling of the samples it was found that a higher efficiency and a higher concentration can be obtained by using the fluorescent labeling kit by PCR method, Cy3 PCR Labeling Kit from Jena Bioscience which uses the incorporation of fluorescently labeled Cy3-dUTP nucleotides during PCR reaction. , resulting in an amplicon containing several fluorescently labeled nucleotides with CY3 fluorochrome. The PCR reaction was performed on the BioradiCycler equipment.

For the PCR reaction, specific primers for the HPV16 strain were used:

Forward Primer: CGC ACA AAA CGT GCA TCG GCT ACC with length 24 bp and 73°C annealing temperature.

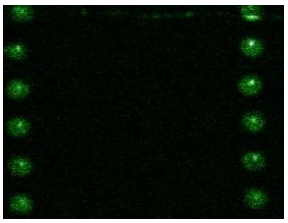
Reverse Primer: TGG GAG GCC TTG TTC CCA ATG GA with length 23 bp and 72.3°C annealing temperature.

The amplicon resulting from the amplification reaction having a length of 217 bp and incorporated inside its dUTP-CY3.

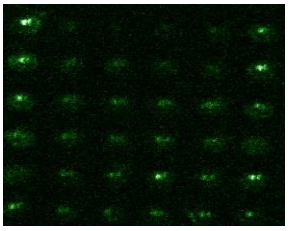
An electrophoresis in agarose gel was performed gel for PCR verification

Electrophoresis *for amplicon with length 217bp.*

Targeted microchips for samples tested for HPV16 by microarray technology had built-in test leads for positive control and identical replicates of different concentrations with specific HPV16 probes.



Images with fluorescent probes used as control probes



Blade images with hybridized fluorescent DNA with complementary probe immobilized on the slide

The columns in this 2 images (left right) are the immobilized the fluorescent controls, and in the 4 middle columns it is observed how the hybridized single-stranded DNA of length 217b from the 217bp amplicon hybridized.

The results obtained by microarray technology were validated by RT-PCR and Sanger sequencing.

Preliminary testing of prenatal diagnostic devices for the detection of HPV infection in real samples will be performed by the inclusion by the IOMC partner of 140 patients at risk of premature birth. The inclusion of patients in the study was achieved in compliance with the national and international ethical norms in force. The samples were taken and processed in order to extract the DNA by the partners.

Results Dissemination:

- "*The influence of molecular weight of ssDNA-SRY and BSA on SPR signal amplification"*, Elena Constantin, Melania Popescu, Monica Simion, EuroNanoForum 2019
- *"Label free detection of protein using SPR signal*", Elena Constantin, Melania Popescu, Monica Simion, NN 2019 - 16th International Conference on Nanosciences & Nanotechnologies, 2019
- *"Enhancement of SPR signal using gold nanorods"*, Elena Constantin, Melania Popescu, Monica Simion, Adina Boldeiu, Iuliana Mihalache, International Semiconductor Conference (CAS), Sinaia, 9-11 Oct., 2019
- *„Microarray flexible platform manufactured on cotton fabrics coated with ultrathin ZnO layer"*, Melania Popescu, Florin Nastase, Elena Constantin, Monica Simion, Cosmin Romanitan, Marian Popescu, Conference of the Romanian Electron Microscopy Society - C.R.E.M.S., 23-25.10.2019, Poiana Brasov