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Paul Moustiez, Aline Cournut, Dmitri Yarekha, Sophie Halliez, Claudia Bich, Morvane Colin, Christine Enjalbal, Yannick Coffinier

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# Elaboration of nanoneedle arrays via nanosphere lithography and single step continuous ICP etching. Application for detections of neurodegenerative diseases' biomarkers by mass spectrometry

P. Moustiez<sup>\*a</sup>, A. Cournot<sup>b</sup>, D. Yarekha<sup>a</sup>, S. Halliez<sup>c</sup>, C. Bich<sup>b</sup>, M. Colin<sup>c</sup>, C. Enjalbal<sup>b</sup>, Y. Coffinier<sup>a</sup>,

<sup>a</sup> IEMN, Villeneuve d'Ascq, 59650, France

<sup>b</sup> IBMM, Montpellier, 34293, France

<sup>c</sup> LiLNCog, Lille, 59000, France

\*e-mail: [paul.moustiez@iemn.fr](mailto:paul.moustiez@iemn.fr)

Silicon nanostructures like nanoneedle arrays present a huge potential for various applications such as photovoltaic cells [1], sensors [2], information storage [3] to name a few. Nanoneedles (NNs) are defined as nanomaterials presenting high aspect ratio. Those belong to two main classes: single needles, externally manipulated to contact cells and tissues (near field microscope (AFM), Micromanipulator) or arrays of vertical high aspect ratio nanostructures supported on a substrate. The former encompasses a wide variety of nanostructures including nanowires, nanopillars, porous nanocones, nanotubes, and nanostraws. Variety of materials/dimensions/shapes make each type of NNs having different properties that befit specific sensing needs, that is to say various applications in mechanobiology, nanoelectrophysiology, optogenetic, nanophotonic, transfection/vectorization (drug delivery) [4].

What we suggest here is the development of new, minimally invasive nanoneedle based platforms for monitoring living cells by leveraging the ability of NNs to access the cell cytoplasm and to get new insights in the understanding of biochemical processes occurring within cells (Figure 1). As a proof of concept, we want to assay the state of intracellular tau in brain cells. Specific modifications and conformational changes of tau are features associated with a group of neurodegenerative disorders called tauopathies, such as Alzheimer's disease (AD). Therefore, assaying intracellular tau state in living cells could allow a better understanding of pathophysiological mechanisms involved in AD and other tauopathies but also be used as a biomarker per se. Of note, extracellular deposition of A $\beta$  peptides is another feature of AD. To this extent, we present the fabrication techniques of the NN's. Following the uniform self-assembly of polystyrene particles via nanosphere lithography step, a single step continuous ICP etching was achieved. The interest of this lithography method is to reduce the cost and increase the speed of fabrication compared to common lithography based processes.

The nanoneedle arrays obtained with this method were then characterized with water contact angle, AFM, SEM and reflectometry measurements. The purpose of these NNs platforms is to combine them with SALDI-MS1 detection; as opposed to MALDI-MS2, this technique doesn't use any matrix but is based on an optimized nanostructured surface such as our NNs which could provide better result in terms of S/N ratio. With that in mind, we developed the surfaces adjusting their characteristics by tuning the fabrication's parameters. Moreover, we were able to functionalize them by silanization or also thanks to electroless metal deposition such as copper, gold, silver (Figure 2) or platinum. SALDI tests were accomplished simultaneously with SERS analysis to be able to confront the results. Finally, we obtained the first results of the effect of the interaction between the needles and primary neural cells in terms of cell viability.

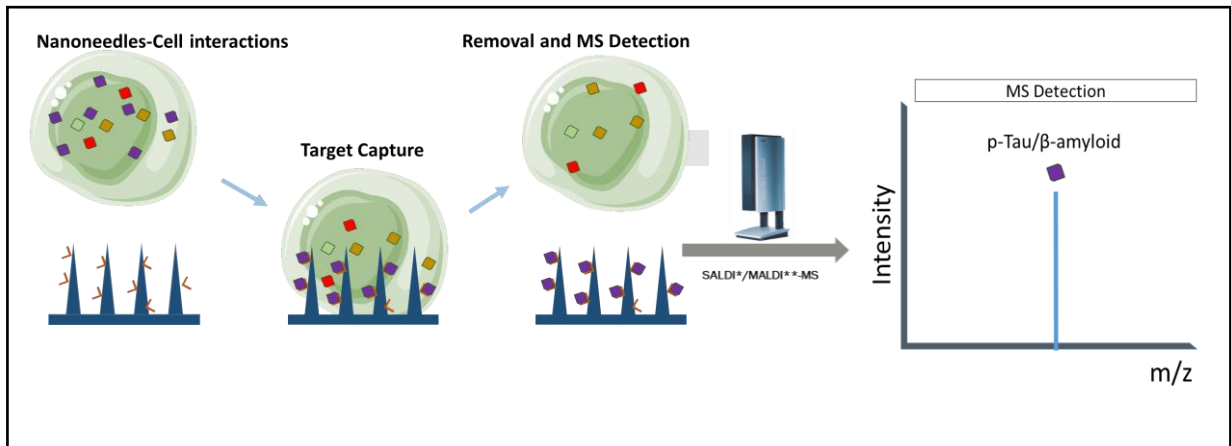
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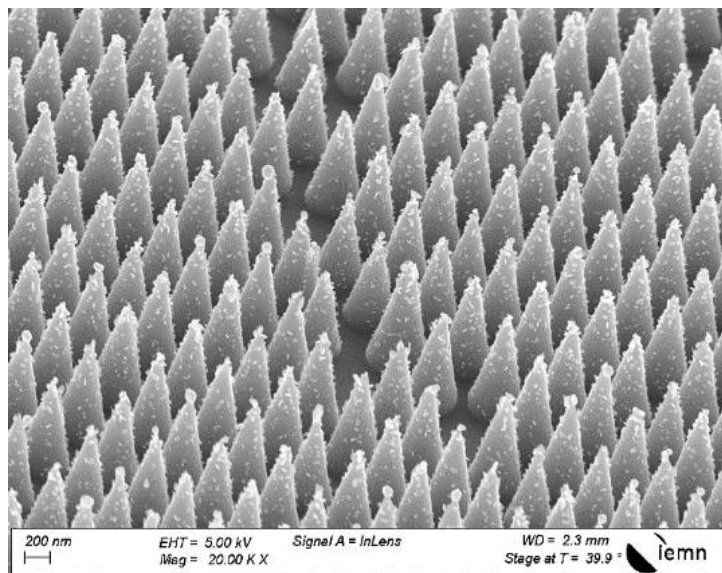
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<sup>1</sup>: Surface Assisted Laser Desorption Ionization - Mass Spectrometry

<sup>2</sup>: Matrix Assisted Laser Desorption Ionization



**Figure 1:** schematic description of the sensing process, left: capture of the target without altering the integrity of the cell, right: analyse of the targets captured by SALDI-MS detection



**Figure 2:** Silver nanoparticles deposited by electroless metal deposition on a nanoneedle array