SYNTHETIC FINAL RESEARCH REPORT 2015-2017

PROJECT CODE: PN-II-RU-TE-2014-4-0862 CONTRACT NO: 381/10-11-2015

Report on project achievement in the period November 10th, 2015 – November 9th, 2017

O1). A) The optimization of the detection method was carried out by testing several SERS-active substrates, different growth conditions for the pathogens, various laser lines for excitation, etc. The most reproducible and reliable SERS results were obtained by using the 532 nm laser line, low power and the **Bacteria@AgNPs approach** for the silver colloid synthesis. The label-free character of the developed detection protocol was assured by the unspecific receptor immobilization procedure of the tested microorganisms. In this regard, the sample supports used were microscope adhesion slides, which are chemically covered with a layer of poly-L-lysine (**polyslides**).

B) By using the label-free SERS detection optimized protocol, the SERS spectral database was built up and is now available on the project's webpage. It contains the specific SERS fingerprints of **25 pathogenic bacterial strains**, both Gram-positive and Gram-negative, including new specimens, as mycobacteria and fungi. The relevant spectral domain from the whole SERS profile, which contains the main SERS marker bands, was determined in order to be analyzed by multivariate analysis (**Principle Component Analysis - PCA**) for unbiased classification and discrimination of the investigated pathogens.

O2). A **PCA algorithm for accurate discrimination** (>80 % specificity and sensitivity) of SERS single-cell bacterial fingerprints was optimized. The developed PCA model was applied on both Gram-positive and Gram-negative species and inter-species as well as intra-species discrimination was successfully performed.

O3). The bacteria SERS detection at single-cell level in **real-life samples** was assessed by using artificial urine samples spiked with bacteria. Sensing the presence of two pathogens found simultaneously in the same sample was also carried out. Moreover, the identification and the discrimination between the two species was possible by applying the SERS-PCA analysis.

O4). The effect of old and new antibiotic formulas was monitored by using the Bacteria@AgNPs approach and ultimately the detection method was validated by obtaining the fast and reliable antibiogram. The SERS spectra of the treated bacteria revealed a specific profile in the case of resistance or sensitivity for antibiotics. Moreover, the first PC's coordinates in the 2D PCA space were used as an indicator of the pathogen's behavior during antibiotic treatment.

Published papers: Dina, N.E., et al., Analyst, **142,** 10, 1782-1789, 2017.

Dina, N.E., et al., J Raman Spec, **48**, 8, 1122-1126, 2017.

Dina, N.E., et al., Crit Rev Anal Chem, 1-14, 2017.

Colniță, A., et al., Nanomaterials (Basel), **7,** 9, 2017.

Project Manager, Dr. Nicoleta Elena Dina