SUBNANOMOLAR DETECTION OF TUBERCULOSIS BIOMARKER MPT64 IN SANDWICH SERS IMMUNOASSAY ON NOVEL LOW-COST SUBSTRATE

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Introduction. The use of SERS for detection, analysis and imaging has attracted great interest in the past decade owing to its high sensitivity and molecular fingerprint specificity. There is an increasing interest in scientific community to SERS as it becomes a versatile method for early medical diagnostics and reliable detection of major health threats to humans (e.g. cancer, tuberculosis, etc) and animals [1]. The key component of our SERS-based immunoassay include:1) a capture substrate to specifically adsorb antigens from solution; (2) Extrinsic Raman Labels (ERLs): surface functionalized gold nanoparticles (AuNPs) to bind to captured antigens selectively and generate intense SERS signals, which contain both capture antigen and SERS active readout molecule (4-nitrobenzenethiol (4-NBT)).

Materials and methods. ERLs preparation used 60nm AuNPs, monoclonal antibodies (MC), 0.5 mM NBT solution. The capture substrate was formed using aluminum foil as the support surface. For preparation of Al substrate cut 1x1 cm glass slide, attach Al foil with a double-sided scotch. Each assay employed the same monoclonal antibody for antigen capture on the substrate and detection with ERLs. Before applying of prepared solutions use parafilm to limit spreading of the drop (Fig. 2). Raman spectra were recorded with Raman Microscope LABRAM Horiba with 785 nm excitation. Intensity was calculated using 1336 cm^{-1} characteristic N-O vibration in NBT molecule.

Fig. 1.
Sample address prepared with parafilm

Results and discussion. From the data obtained by the calculation Raman spectra (Fig. 1) limit of detection was determined. This LOD corresponds to average of 4-6 molecules in the focused laser beam area, producing signal above 3 standard deviation of the blank signal. Limit of detection (LOD) for MPT64 (tuberculosis biomarker) with monoclonal antibodies on Al foil is ~ 0.2 nM (to be reproduced when new chemicals will be delivered)

Conclusions. As a proof of principle, just in the first attempt, we achieved sub 1 nM LOD for detection of tuberculosis marker MPT64 using SERS immunoassay with Al foil as the bottom substrate, even as it is likely to be the first application of this high sensitivity biodetection method in Kazakhstan. As soon as we obtain MPT64 antibodies we are planning to reproduce and improve our results in MPT64 SERS immunoassay. Collaborating with a group of Dr. Kanayeva we are planning to test aptamer based SERS assays for detection of MPT64 and possibly a breast cancer biomarker.

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References.

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