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SERS for biomedical studies

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Introduction: Noninvasive medical diagnostics for screening and personal medicine face a challenge of highly sensitive single molecule or single cell analysis. This demands thorough development of new materials and novel analytical approaches. In this context, Raman Scattering Spectroscopy (RSS) is especially suited for the detection of various molecule fingerprints, biomolecule conformation and interactions in biological samples, even in living cells. The disadvantage of RSS is relatively low intensity of Raman scattering of most of biological objects. Surface Enhanced Raman Spectroscopy (SERS) allows to detect biomolecules in submicromolar concentrations when they are located in the close vicinity (<15-20 nm) to the surface of nanostructures, usually silver or gold. Though Ag or Au colloids are widely used in SERS, nanostructured substrates promise much better reproducibility of Raman scattering enhancement and less cell toxicity that promote their applications in biology and medicine, especially in the development of highly sensitive lab-on-a-chip devices. For the first time we report application of Nanostructured Silver Substrates (NSS) with the “coffee ring” morphology for SERS studies of living erythrocytes and functional mitochondria.

Materials and methods: Experiments were done on rat and human erythrocytes and isolated mitochondria from the rat heart. Nanostructured surfaces were fabricated by ultrasonic deposition of aqueous diamminesilver hydrochloride on glass substrate.

Results and discussion: We demonstrate that proposed NSSs do not evoke erythrocyte hemolysis and that their “coffee ring” morphology results in the immobilization of erythrocytes, providing a tight contact of NSS with erythrocyte plasma membrane. As a result, SERS spectra from submembrane hemoglobin inside single living erythrocytes were recorded, confirming the effectiveness of the proposed NSSs for the design of biosensor chips. We were also first to observe SERS spectra of cytochrome c in intact functioning mitochondria placed on proposed NSS. We demonstrated that SERS spectra of cytochrome c in mitochondria are sensitive to the mitochondrial membrane potential and to ATP production in ATP-synthetase.

Conclusion: The proposed SERS-based approach can be used for fundamental studies of functional and conformational properties of cytochrome c in intact mitochondria and it is promising in clinical applications on diagnostics of mitochondrial dysfunction.