Antithrombotic and antimicrobial activity of functionalized silver nanoparticles performed under in vitro, ex vivo condition and in animal model

Despite the significant progress in understanding of the thrombosis pathophysiology, thromboembolic disease and its complications still are a major worldwide cause of death and disability. While there are medications that reduce the risk of unwanted activation of the blood coagulation system, their effectiveness is sometimes inadequate and furthermore they increase the risk of bleeding. Therefore, researchers all over the world continuously search for new antithrombotic drugs with increased efficacy and reduced side effects. The unique properties of pharmaceutical materials in *nano* scale i.e. large surface area to size/volume, the ability to cross biological membranes in conjunction with nanoparticle functionalization allow decreasing drug doses and increasing selectivity and efficacy of pharmacotherapy, which in turn reduce side effects and cost therapy. When it comes to the nanopharmaceutical development of antithrombotic drugs, the field still has not been explored yet. The research in collaboration with Professor Radomski team demonstrated that functionalized silver nanoparticles (AgNPs) not only inhibit platelet aggregation stimulated by various agonists but also disaggregate platelets. Therefore, the scientific purpose of the project is a comprehensive analysis of the impact of AgNPs on hemostasis depending on the particle size, physicochemical properties and structural modifications, that will help elucidate the anticoagulant mechanism. Moreover, in our study we are going to modify surface of polyurethane catheter with functionalized AgNPs, carefully selected during performed experiments, and evaluate their ability to prevent blood clotting, bacterial infection, adhesion and biofilm formation. Therefore, we have designed the multidirectional studies conducted on cellular lines, blood platelets taken from healthy volunteers and animal models of thrombosis to investigate the comprehensive influence of silver nanoparticles on hemostasis (blood platelets, endothelium cells, fibrinolysis). In our study we will use: transmission and scanning electron microscope Quanta SEM FEI, atomic force microscopy Nanosurf AFM and INTEGRA, He-ion; the zeta potential analyzer and Nanosight, flow cytometry, electrochemical nanosensors for NO, $NO/ONOO^-$ ratio detection in biological systems. It is necessary to emphasize that we will evaluate the platelet-silver nanoparticles near physiological conditions: in real time under flow condition and with nanoresolution using unique method based on quartz crystal microbalance with dissipation (QCM-D). We have found that QCM-D is able to measure nanoparticle-induced platelets microaggregation at concentration that were undetectable by light aggregometry and flow cytometry.

Our research will contribute significantly to the expansion of the general biomedical knowledge and understanding of the AgNPs action with potential antithrombotic properties. There results will be of significant importance for the development of nanopharmacology and nanotoxicology, not to mention potential application in medicine. The combination of the ability to inhibit blood platelets activity and antibacterial properties may cause that AgNPs will become an ideal material for manufacturing biological implants or drug carriers. The effects of disaggregation combined with small size, can bring new perspectives for the treatment of thrombosis/microclots in the brain, eye, lungs, or other organs.