Scientific Memo

To: Doctoral School , University of Medicine and Pharmacy "Carol Davila" of Bucharest
From: **Christien Oktaviani MATEI**
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Thesis title: ***CONTRIBUTION OF BIOPHYSICAL METHODS IN EVALUATION OF CELLULAR PARAMETERS IN PATHOLOGIC CONDITIONS AND NANOPARTICLE THERAPIES***

Introduction

My doctoral thesis offers a comprehensive and interdisciplinary contribution to the fields of hematology malignancies and cancer nanomedicine by applying advanced biophysical methods to investigate how cellular parameters are altered in both pathological conditions and in response to therapeutic interventions. The work is structured around two major experimental studies: the first focusing on platelet biophysics in hematological patients, and the second assessing the cytotoxicity and biocompatibility of engineered nanoparticles in normal and cancerous cell lines.

The methodological innovation presented in Study 1 centers on the development and optimization of a panel of fluorescence-based assays for use with small-volume platelet samples. Traditional platelet function assays often require large sample volumes and are typically limited to single-parameter evaluations. By contrast, my approach enables simultaneous assessment of resting membrane potential (RMP), membrane fluidity, and reactive oxygen species (ROS) levels—three critical indicators of platelet function and mitochondrial activity. This integration of multiparametric biophysical data, applied directly in a clinical research setting, represents a novel contribution to the field of platelet diagnostics.

The biophysical analysis conducted on platelets from CLL patients revealed a consistent pattern of altered function, particularly in those receiving ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor widely used in clinical practice. Specifically, platelets from ibrutinib-treated patients exhibited elevated ROS levels, increased membrane fluidity, and significantly depolarized RMP compared to healthy controls. These findings provide a plausible mechanistic basis for the known bleeding complications associated with ibrutinib therapy and suggest that monitoring platelet biophysics could serve as a predictive tool for treatment risk management.

In parallel, Study 1 analyzed platelets from patients with MPNs, including essential thrombocythemia, polycythemia vera, and myelofibrosis—disorders characterized by dysregulated proliferation of myeloid lineages and an increased risk of thrombosis. The biophysical assays revealed that platelets from thrombotic MPN patients displayed hyperpolarized membrane potentials, a finding rarely reported previously in the literature. Moreover, this alteration appeared to normalize in patients receiving cytoreductive therapy, indicating a dynamic and treatment-responsive phenotype. Notably, the study also showed

that aspirin modulates membrane fluidity, providing new insight into the biophysical actions of this common antiplatelet drug.

Together, these results highlight the capacity of biophysical assays to detect subtle, yet clinically meaningful, changes in platelet function. Such assays could complement conventional coagulation tests by providing real-time, functional readouts of platelet health, particularly in oncology patients who are at dual risk of thrombosis and bleeding. The ability to simultaneously monitor multiple functional aspects of platelets offers a powerful tool for precision medicine approaches in hematological malignancies.

The second half of my thesis transitions into the field of nanomedicine, specifically evaluating the safety and therapeutic potential of metal-oxide nanoparticles in cell culture models. I investigated manganese-doped zinc oxide (ZnO:Mn) nanoparticles and iron/titanium oxide (Fe_3O_4/TiO_2) nanocomposites—both materials with established or emerging applications in oncology, drug delivery, and imaging. These nanoparticles were synthesized and characterized with the amiability of IFIN Magurele scientific collaborators by using advanced physicochemical techniques including X-ray diffraction (XRD), transmission electron microscopy (TEM), electron paramagnetic resonance (EPR), and Fourier-transform infrared spectroscopy (FTIR).

The ZnO:Mn nanoparticles demonstrated notable cytotoxic effects on cancer cell lines while exhibiting lower toxicity in normal fibroblasts, particularly at moderate Mn doping levels. Notably, increased Mn content was associated with reduced ROS generation and DNA fragmentation in healthy cells—suggesting a protective, dose-dependent effect of manganese doping. These findings contribute to a growing body of evidence that Mn-doping can attenuate the pro-oxidant effects of ZnO, thus improving its therapeutic index and biocompatibility.

The research underscores the importance of assessing nanoparticle safety not only through viability assays but also via detailed biophysical analyses. Parameters such as ROS generation and membrane integrity (assessed through LDH release) provide mechanistic insights into cytotoxic pathways and help identify safe dosage ranges. Furthermore, the thesis highlights the need for integrated testing platforms that combine biophysical, biochemical, and imaging modalities to comprehensively evaluate nanomaterial behavior in biological systems.

The original contributions of my thesis are thus multifold. On the methodological front, I propose a new standard for platelet analysis in clinical research, combining sensitivity, efficiency, and multiparametric depth. On the biological side, the studies provided novel insights into disease- and treatment-specific alterations in platelet biophysics, revealing previously undocumented effects of therapies like ibrutinib and aspirin. In cancer nanomedicine, I reported the comparative toxicological profile of doped versus undoped ZnO nanoparticles, and described how Mn doping reduces oxidative damage in normal cells. The synthesis and cellular evaluation of Fe/TiO_2 nanocomposites further expand the understanding of how nanostructured materials interact with living systems.

In the final chapter, my thesis outlines several future directions. It suggests that larger, longitudinal clinical studies are needed to validate platelet biophysical parameters as predictive biomarkers for thrombotic and bleeding risk. Additionally, further exploration into the mechanisms underlying Mn-mediated reductions in nanoparticle toxicity is warranted—potentially involving investigations into particle dissolution, intracellular uptake, and ROS scavenging pathways. My thesis also recommends testing these nanomaterials in more complex biological models, including co-cultures and in vivo systems, to evaluate their translational potential.

Research Objectives

1. Study 1 aims to assess biophysical parameters (membrane potential, fluidity, ROS, ATP, and aggregation) in platelets from MPN and CLL patients to identify disease-related alterations.

2. Study 2 evaluates the effects of ZnO:Mn and Fe_3O_4/TiO_2 nanoparticles on normal and cancerous cells using assays for viability, ROS production, and DNA fragmentation to determine therapeutic impact.

Key General Achievements

Developed and optimized protocols for evaluating RMP, membrane fluidity, ROS, and platelet aggregation.

Design and execution of all nanoparticle exposure experiments across murine and human cell models.

Performed data analysis for viability, ROS, DNA damage, and LDH assays.

Synthesis and validation of findings into a framework connecting biophysical properties with therapeutic impact and safety in nanomedicine

Study Specific Achievements

Study 1- Platelet Biophysics in Hematologic Disorders

Multiparametric analysis revealed that platelet dysfunction in MPNs, CLL, and thrombotic conditions involves altered membrane potential, redox imbalance, and chemotherapy effects.

- Platelets from MPN patients, especially those with thrombosis, showed RMP hyperpolarization and elevated ROS, with chemotherapy (e.g., hydroxyurea) partially normalizing these parameters.
- ASA modified membrane fluidity independently of COX-1, potentially contributing to its antithrombotic action.
- In CLL, ibrutinib-treated patients had more fluid platelet membranes, lower RMP, and increased ROS, correlating with bleeding risks.
- Not all dysfunctions were membrane-driven, suggesting a role for receptor-level changes.

- RMP and ROS may serve as potential biomarkers for thrombotic risk and treatment response.

Study 2 – Nanoparticle Cytocompatibility and Stress Response

Biophysical assays were optimized to assess cytotoxicity and oxidative stress from Mn-doped ZnO and Fe/TiO_2 nanomaterials.

- ZnO NPs synthesized with PVP or SHMTP exhibited surfactant- and doping-dependent size and surface properties.
- Cell viability remained high at ≤10 µg/mL across all ZnO NPs, with SHMTP-based NPs showing reduced ROS and DNA damage due to higher Mn incorporation.
- Fe/TiO_2 nanocomposites induced mild, Fe-dependent cytotoxicity in NIH 3T3 cells, while HS27 fibroblasts showed high tolerance and membrane integrity.
- Cell-free MTS assays confirmed minimal interference, validating the assay approach.
- The results underline the importance of nanoparticle structural design—Mn doping and surfactant choice were key to improving biocompatibility.

Publications and Dissemination

A. Peer-Reviewed Papers:

1. Popov V, Matei CO, Omer M, Matei M, Savopol T, Bumbea H, Moisescu MG, Ibrutinib on biophysical parameters of platelet in patients with chronic lymphocytic leukaemia,
American Journal of Blood Research, 2020, 10, 311–319, FI - 2.6/2020, Q3, capitolul IV, pag. 77–85 https://www.ajblood.us/files/ajbr01000311.pdf
(This article is covered in chapter 6.3.2 CLL patients without thrombosis)

2. Popescu T, Matei CO, Vlaicu ID, et al.,Influence of surfactant-tailored Mn-doped ZnO nanoparticles on ROS production and DNA damage induced in murine fibroblast cells,Scientific Reports, 2020, 10, 18062, FI - 4.379/2020, Q1, capitolul V, pag. 93–104https://doi.org/10.1038/s41598-020-74816-0 (This article is covered in chapter 7.3.2. Experiment 2 : Zinc Oxide Nanoparticles biocompatibility analysis)

3. Popescu T, Matei CO, Culita D, Maraloiu VA, Rostas A, Diamandescu L, Iacob N, Savopol T, Ilas M, Feder M, Lupu A, Iacoban A, Vlaicu I, Moisescu MG, Facile synthesis of low toxicity iron oxide/TiO2 nanocomposites with hyperthermic and photo-oxidation properties,
Scientific Reports, 2022, 12, Article 11003, FI - 4.996/2022, Q1, capitolul V, pag. 105–116
https://doi.org/10.1038/s41598-022-11003-3 (This article is covered in chapter 7.3.3. Experiment 3: Fe/TiO2 Nanocomposite biocompatibility analysis)

4. Matei CO, Popov VM, Matei MB, Kovacs E, Moisescu MG, Savopol T,

Membrane potential evaluation of platelets from chronic myeloproliferative neoplasms and thrombosis patients, Romanian Journal of Physics, 2025, 70(2), in press, FI - estimat 0.7/2025, Q4, capitolul IV, pag. 86–92)

B. Oral presentation

The biocompatibility of Iron oxide-TiO2 nanocomposites with magnetic and photocatalytic properties. Christien Oktaviani Matei, Traian Popescu, Ioana Dorina Vlaicu, Tudor Savopol, Mihaela Georgeta Moisescu. Prezentare orala: în cadrul Sesiumii Tânărului Cercetător - Specialităţi preclinice - la congresul universităţii de medicină şi farmacie "carol davila" din bucuresti, editia A IX-A. desfăşurat online în perioada 25-27 noiembrie 2021.

Conclusion

In conclusion, this thesis demonstrates the translational potential of biophysical methods in two key biomedical domains: characterizing platelet dysfunction in hematological cancers and evaluating the safety and therapeutic prospects of nanomaterials in oncology. The findings confirm the central hypothesis that biophysical assays can capture clinically relevant changes in cell behavior, offering tools that are both scientifically rigorous and practically valuable for diagnostics, prognostics, and therapeutic development. These contributions have been disseminated through multiple scientific publications that are actively cited, attesting to their originality and relevance.

Signature:
[Christien Oktaviani Matei]