"CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST

DOCTORAL SCHOOL

MEDICINE

CONTRIBUTION OF BIOPHYSICAL METHODS IN EVALUATION OF CELLULAR PARAMETERS IN PATHOLOGIC CONDITIONS AND NANOPARTICLE THERAPIES

SUMMARY OF THE DOCTORAL THESIS

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CONTENT

Introduction

Recent advances in live-cell imaging and biophysical methods have improved our ability to study cellular structure and function in real time, deepening insight into both normal and pathological processes. This thesis explores cellular biophysics in the context of hematologic malignancies and nanomedicine through two studies. The first examines platelet function in chronic lymphocytic leukemia (CLL) and myeloproliferative neoplasms (MPNs), where altered biophysical properties may serve as diagnostic markers. The second evaluates the effects of ZnO:Mn and Fe_3O_4/TiO_2 nanoparticles on healthy and cancerous cells by measuring membrane potential, fluidity, ROS, ATP, and DNA damage. Together, these studies highlight the role of biophysical tools in disease characterization and therapeutic development.

I. GENERAL OVERVIEW

1. Biophysical methods for evaluation of cellular parameters relevant to studies 1 and 2

Cellular health and function can be assessed through key biophysical parameters such as membrane potential, fluidity, reactive oxygen species (ROS), ATP content, viability, aggregation behavior, and DNA integrity [1]. The resting membrane potential, maintained by ion channels and transporters, reflects cellular excitability and is measured using techniques like patch clamp or voltage-sensitive dyes [2]. Membrane fluidity, essential for protein function and signaling, depends on lipid composition and is commonly analyzed by fluorescence-based methods [2]. ROS, though important in signaling, cause oxidative damage when elevated and are detected via fluorescent probes or chemiluminescence [3,4]. ATP assays indicate metabolic activity, with luminometric luciferase-based methods offering high sensitivity [5,6]. Cell viability is evaluated through membrane integrity, metabolic assays (e.g., MTT, Alamar Blue), or cytotoxicity markers like LDH release [7]. Aggregation tests assess the clustering behavior of cells or particles—used in platelet function analysis [8] or nanoparticle stability studies—while DNA fragmentation assays (e.g., comet, TUNEL) reveal genomic damage linked to apoptosis or genotoxic stress [9]. Together, these

measurements provide comprehensive insights into cellular physiology, pathology, and responses to external stimuli.

2. Platelets structure and functions; pathological conditions

Platelets are small, anucleate cell fragments (2–5 μm) derived from megakaryocytes, playing central roles in hemostasis, inflammation, and vascular repair [8]. Upon vascular injury, they activate rapidly, release granules, and aggregate through cytoskeletal remodeling [10]. Beyond primary hemostasis, platelet dysfunction is a key contributor to pathological thrombosis, particularly in chronic lymphocytic leukemia (CLL) and myeloproliferative neoplasms (MPNs) In CLL, impaired B lymphocytes and targeted therapies—such as Bruton's tyrosine kinase (BTK) inhibitors like ibrutinib—disrupt platelet signaling and increase bleeding risk despite preserved platelet counts [11]. In MPNs, driver mutations (e.g., JAK2, CALR, MPL) lead to myeloid proliferation and a high thrombotic burden due to altered platelet function and hypercoagulability [12]. Chemotherapeutic agents such as hydroxyurea and JAK inhibitors (e.g., ruxolitinib) reduce thrombotic risk by modulating platelet count, redox state, and membrane potential [13]. Acetylsalicylic acid (ASA) remains a cornerstone of antithrombotic therapy through irreversible COX-1 inhibition, suppressing thromboxane A_2-mediated aggregation [14]. Thus, integrating platelet biophysical profiling with therapeutic strategies offers potential to improve thrombosis management and personalize treatment in hematologic malignancies.

3. Zinc oxide nanoparticles and iron oxide/ titanium dioxide nanocomposites

Zinc oxide (ZnO) nanoparticles and iron oxide/titanium dioxide (Fe_3O_4/TiO_2) nanocomposites represent two promising nanomaterial platforms in biomedicine, each offering distinct physicochemical and functional advantages [15]. ZnO nanoparticles, synthesized through sol-gel, hydrothermal, or green methods, exhibit high surface area, photoluminescence, and selective cytotoxicity, enabling their application in bioimaging, drug delivery, wound healing, and cancer therapy [16]. Their biological activity is largely mediated by reactive oxygen species (ROS) generation, Zn^{2+} ion release, and membrane disruption, though challenges remain in modulating toxicity and preventing aggregation. In parallel, Fe_3O_4/TiO_2 nanocomposites combine the magnetic responsiveness of iron oxide

with the photocatalytic and biocompatible properties of TiO_2, allowing for multifunctional applications including MRI, hyperthermia, drug delivery, and antibacterial therapy [17]. These composites enable precise localization and light-activated therapeutic responses but require further optimization for near-infrared (NIR) activation, scalable synthesis, and long-term biocompatibility. Together, these nanostructures underscore the potential of engineered materials in theragnostic, while highlighting the importance of standardized evaluation and in vivo validation for safe clinical translation.

II. PERSONAL CONTRIBUTIONS

4. Working hypothesis and general objectives

This thesis explores the intersection of cellular biophysics, hematologic malignancies, and nanomedicine through two experimental studies.

The general objective of this study is to demonstrate the utility of biophysical methods in detecting functional changes in platelets from MPN patients and in evaluating cellular responses to nanoparticle therapies.

- 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.
- 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.

5. General research methods

This study involved two experimental models. Study 1 included healthy volunteers and patients with hematologic conditions (thrombosis, CLL, MPNs) from Colentina Hospital, Bucharest, with informed consent and ethics approval. Platelet parameters—RMP, MF, and ROS production were measured using fluorescence spectrophotometry, while ATP content using chemiluminescence, and aggregation test using light transmission aggregation (LTA). Study 2 used ZnO:Mn and Fe_3O_4/TiO_2 nanoparticles synthesized in collaboration with the National Institute of Materials Physics. Standardized cell lines were cultured under 2D conditions, and biophysical assays (MTS, LDH, ROS, comet) assessed nanoparticle effects.

Data was processed using Excel, SPSS v27, Phyton and Origin 8.0. Normality was evaluated by Shapiro–Wilk and Kolmogorov–Smirnov tests, with appropriate parametric or nonparametric statistical methods applied. Significance was set at $p < 0.05$.

6. Study 1: Platelets condition in chronic lymphocytic leukemia, myeloproliferative neoplasms, and thrombotic disorders

Platelet membrane biophysical properties, including resting membrane potential (RMP), membrane fluidity (MF), and reactive oxygen species (ROS) production, are critical for platelet activation, aggregation, and function. In hematologic malignancies such as myeloproliferative neoplasms (MPNs) and chronic lymphocytic leukemia (CLL), as well as thrombotic disorders, both quantitative and qualitative platelet abnormalities contribute to thrombotic and hemorrhagic complications [13]. MPNs involve clonal hematopoietic proliferation driven by mutations (e.g., JAK2 V617F), while CLL is associated with platelet dysfunction, particularly under treatment with Bruton's tyrosine kinase (BTK) inhibitors like ibrutinib. This study investigates platelet alterations in MPN, CLL, and thrombotic patients by assessing RMP, MF, ROS, intracellular ATP, and aggregation function, with a focus on the effects of disease and pharmacological treatments, including ASA and cytoreductive therapies. The aim is to distinguish disease-specific platelet alterations from those induced by thrombotic events or medication.

6.1. Research methods

This study included 261 participants: 55 healthy volunteers and 206 patients from the Cardiology and Hematology Departments of Colentina Hospital, all enrolled with informed consent and ethics committee approval. The control group comprised 55 healthy individuals, 25 of whom were re-evaluated after 14 days of daily low-dose acetylsalicylic acid (ASA, 75 mg) to assess platelet changes over their lifespan. The patient cohort included 18 individuals with thrombotic events unrelated to myeloproliferative neoplasms (MPNs) and 153 patients diagnosed with MPNs, further stratified by thrombosis history and chemotherapy status: MPN-T (no thrombosis, n=109) and MPN+T (with thrombosis, n=44), with subgroups receiving or not receiving chemotherapy. Additionally, 35 chronic lymphocytic leukemia (CLL) patients were included, split into those treated with ibrutinib (n=25) and untreated (n=10), all without thrombosis. Diagnostic classification followed

WHO and IWCLL guidelines, with further stratification by treatment type (e.g., hydroxyurea, ruxolitinib, ibrutinib). Exclusion criteria comprised active infections, other malignancies, major cardiovascular events, or substance abuse[17,18].

Venous blood was collected into sodium citrate tubes with gentle handling to prevent platelet activation. Platelet-rich plasma (PRP) was isolated by centrifugation (130–150 × g, 10 min) and purified via Sepharose CL-2B gel-filtration in calcium-free Tyrode buffer with apyrase (0.2 U/mL). Platelet-poor plasma (PPP) was prepared at 2000 × g (15 min) for use as an aggregation blank. Resting membrane potential (RMP) was assessed using $DiSC_3(5)$ fluorescence, calibrated via valinomycin and potassium titration. Membrane fluidity was measured via anisotropy using TMA-DPH. ROS production was quantified using the DCFDA assay, and ATP levels by chemiluminescence (CellTiter-Glo). Aggregation was evaluated using light transmission aggregometry with PRP exposed to ADP, collagen, epinephrine, or ristocetin. Data were analyzed in SPSS using Shapiro–Wilk for normality, followed by Kruskal–Wallis and median tests ($p < 0.05$), with graphical output from Origin, PowerPoint, and Python-based heatmap correlation.

6.2. Results and discussion

6.2.1. Effect of ASA in healthy control and thrombotic patients

Platelet biophysical profiling revealed distinct alterations associated with thrombosis and ASA treatment (Figure 1). Resting membrane potential (RMP) showed no correlation with age or gender and remained unchanged in healthy individuals after 14 days of ASA administration (−58.2 mV vs. −57.3 mV, $p = 0.885$). In contrast, thrombotic patients exhibited significantly hyperpolarized RMPs (−70.5 mV, **$p < 0.001$), suggesting enhanced platelet excitability.

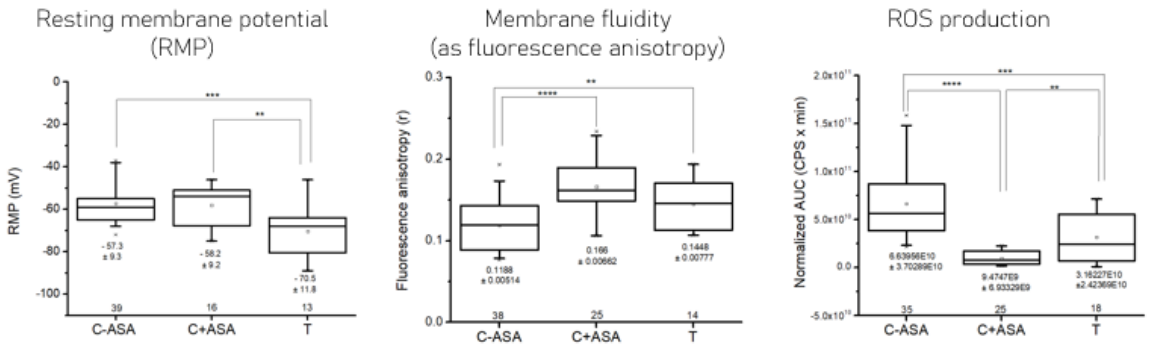

Figure 1 Platelets RMP, MF, and ROS production from control and thrombotic patients associated with thrombosis and ASA treatment

Membrane fluidity, measured by fluorescence anisotropy, was likewise unaffected by demographic factors but significantly decreased after ASA treatment (0.1188 to 0.1660, ***$p < 0.0001$), indicating increased membrane rigidity. Thrombotic patients showed intermediate anisotropy (0.1448), higher than untreated controls ($p < 0.01$), reflecting impaired membrane dynamics.

ROS levels were highest in untreated controls and significantly reduced post-ASA (**$p < 0.0001$), while remaining moderately elevated in thrombotic patients, consistent with ongoing oxidative stress. ASA also impaired ADP-induced aggregation, reducing amplitude, AUC, and increasing disaggregation. Thrombotic patients exhibited further impaired aggregation and delayed responses across ADP, collagen, and epinephrine stimulation, while ristocetin responses remained intact, indicating preserved vWF–GPIb function. Heatmap analysis confirmed weakened responses to key agonists in both ASA-treated and thrombotic groups. These findings suggest that membrane hyperpolarization and reduced fluidity may contribute to dysfunctional platelet signaling and aggregation in thrombotic states. ASA's effects on ROS and aggregation support its role beyond COX inhibition, potentially involving membrane protein acetylation [19]. Collectively, RMP and membrane fluidity emerge as sensitive markers of platelet reactivity and potential therapeutic targets in thrombosis.

6.2.2. CLL patients without thrombosis

In chronic lymphocytic leukemia (CLL), platelet function is influenced by both disease state and ibrutinib therapy, with clear biophysical alterations (Figure 2). Resting membrane potential (RMP) remained stable in untreated CLL patients but was significantly depolarized in those receiving ibrutinib (−42.2 mV vs. −57.3 mV, $p < 0.01$), indicating altered membrane excitability.

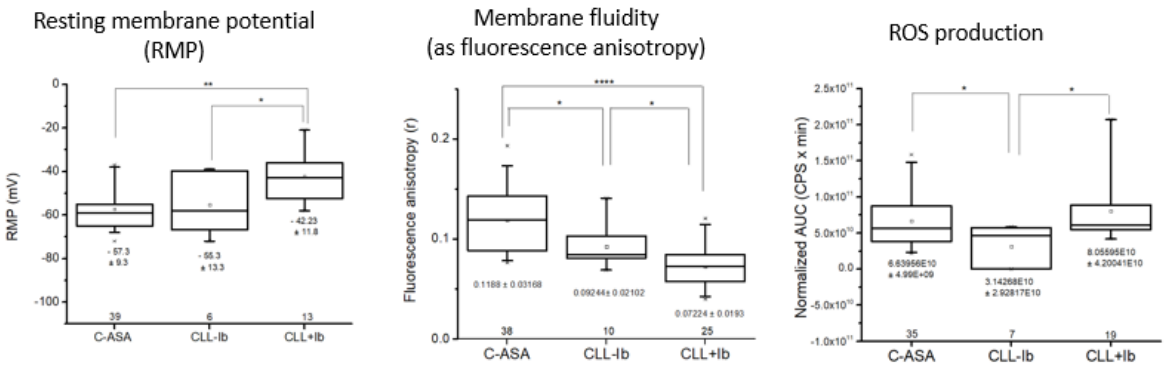

Figure 2 Platelets RMP, MF, and ROS production from control and CLL patients with and without ibrutinib

Membrane fluidity increased in both CLL groups, especially with ibrutinib treatment ($p < 0.0001$), suggesting disrupted lipid organization. ROS levels were reduced in untreated CLL but markedly elevated under ibrutinib ($p < 0.05$), reflecting a therapy-induced oxidative shift. Platelet aggregation was progressively impaired—particularly in response to ADP and collagen—under disease progression and BTK inhibition, while epinephrine responses were delayed and ristocetin responses remained unaffected. These patterns suggest selective dysfunction in BTK- and COX-1-dependent pathways [17]. Biophysically, the triad of membrane depolarization, increased fluidity, and oxidative stress in ibrutinib-treated patients compromises platelet signaling and may contribute to bleeding risk. ROS-driven lipid peroxidation likely alters membrane dynamics, impairing receptor clustering. Despite preserved GPIb-vWF function, aggregation defects highlight the need for biophysical monitoring in CLL to better manage antithrombotic strategies and predict hemorrhagic complications.

6.2.3. MPNs patients with and without thrombosis

Platelets from patients with myeloproliferative neoplasms (MPNs), particularly those with thrombosis (MPN+T) and non-MPN thrombotic patients (T), displayed significantly hyperpolarized resting membrane potentials (RMP) compared to healthy controls (Figure 3), whereas MPN–T patients showed no significant difference [18]. This trend was consistent across MPN subtypes—polycythemia vera (PV), essential thrombocythemia (ET), and unclassified MPNs (UMN)—with the exception of primary myelofibrosis (PMF).

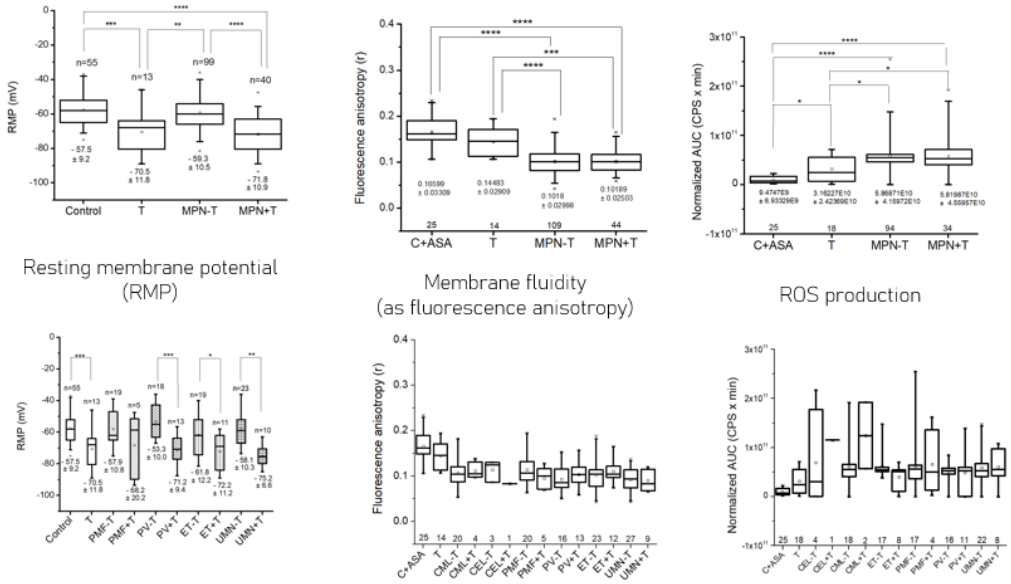

Figure 3 Platelets RMP, MF, and ROS production from control, thrombotic and MPN patients with and without thrombosis

Chemotherapy, especially hydroxyurea, partially reversed RMP hyperpolarization (Figure 4), while kinase inhibitors had no effect [18]. Membrane fluidity was elevated in all MPN groups regardless of thrombosis, with persistent increases in MPN+T even under treatment, indicating ongoing lipid remodeling. ROS levels were uniformly elevated across all MPN subtypes, independent of thrombotic status, with the greatest variability observed in chronic eosinophilic leukemia (CEL) and chronic myeloid leukemia (CML).

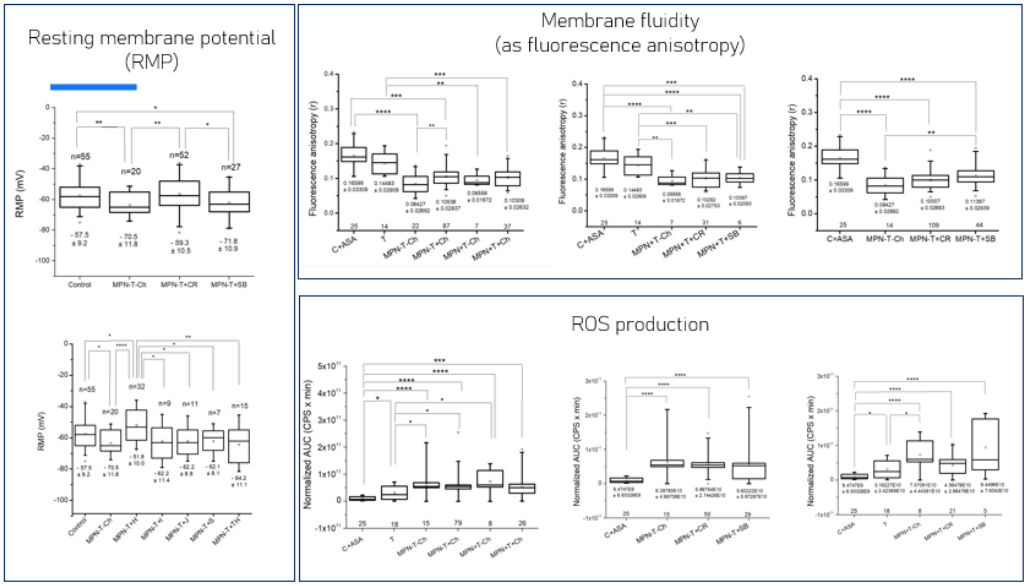

Figure 4 Platelet RMP, membrane fluidity, and ROS levels in controls, thrombotic, and MPN patients, grouped by thrombosis and chemotherapy status

Hydroxyurea and ruxolitinib increased ROS variability, while interferon therapy slightly reduced it. ATP levels positively correlated with RMP, suggesting that RMP shifts result from ion channel or metabolic alterations rather than ATP depletion. ASA treatment elevated ATP levels, aligning with previous reports of enhanced mitochondrial biogenesis and Sirt1 activation. Platelet aggregation was impaired in all MPN groups, showing ADP-induced disaggregation, delayed collagen responses, reduced epinephrine slope, and diminished ristocetin-induced activity. These functional impairments correlated with RMP hyperpolarization and elevated ROS levels.

Overall, MPNs are associated with a constellation of biophysical and functional platelet abnormalities—exacerbated by thrombosis and partially responsive to hydroxyurea—that highlight the interplay between membrane dynamics, oxidative stress, and aggregation dysfunction, underscoring the importance of personalized approaches to managing thrombo-hemorrhagic risk in MPN patients.

6.3. Conclusion

Collectively, these findings reveal that platelet dysfunction in MPNs, CLL, and thrombotic disorders involves hyperpolarized RMP, increased membrane fluidity, elevated ROS, and impaired aggregation, particularly in thrombosis and untreated cases. Hydroxyurea effectively normalizes RMP and ROS, while ASA alters membrane dynamics beyond COX-1 inhibition. Ibrutinib-treated CLL patients show pronounced membrane and oxidative changes. These results suggest that platelet dysfunction is driven more by receptor-level signaling alterations than by intrinsic membrane defects, highlighting the potential of biophysical biomarkers for risk assessment and treatment monitoring.

7. Study 2: Nanoparticles exposure on cell cultures

7.1. Introduction

Mn-doped ZnO nanoparticles were synthesized using PVP and SHMTP surfactants to control shape and size, yielding spherical (~23 nm) and rod-like (~30 nm) structures, respectively [20]. Manganese doping altered the physicochemical properties of ZnO, and the NPs were characterized by XRD, TEM, FTIR, EPR, and surface analysis [16]. Their cytotoxicity was evaluated in murine cell lines by assessing viability (MTS), ROS production, and DNA damage (Comet assay). In parallel, Fe_xO_x/TiO_2 nanocomposites were

developed for potential biomedical applications, with different Fe/Ti ratios forming core–shell or mixed structures. Biocompatibility was tested in normal cells using MTS and LDH assays.

7.2. Research methods

All nanoparticle syntheses and thermal treatments were conducted in collaboration with the National Institute of Materials Physics, Măgurele, Romania. Mn-doped ZnO and Fe_xO_x/TiO_2 nanomaterials were sterilized by autoclaving and dispersed in appropriate culture media to create 2000 µg/mL stock suspensions. To ensure homogeneity, ZnO NPs were sonicated for 1 hour, while Fe/TiO_2 nanocomposites (NCs) underwent 6 hours of sonication. Serial dilutions were used to treat cells pre-seeded 24 hours prior. Cell viability was assessed via the MTS assay, ROS production through H_2DCFDA fluorescence imaging, DNA damage via alkaline comet assay, and cytotoxicity using LDH release quantification. Data were expressed as mean ± SD and analyzed using one-way ANOVA with Tukey's post-hoc test ($p < 0.05$).

The ZnO NP study evaluated six variants (undoped and Mn-doped at 50, 500, and 2000 ppm), stabilized with either PVP or SHMTP, across four cell lines (NIH 3T3, OK, CaCo-2, MCF-7). Cell-free controls confirmed no assay interference, and concentrations ≤10 µg/mL were generally non-cytotoxic. Further testing on NIH 3T3 cells showed surfactant- and doping-dependent effects, with higher Mn content—especially in SHMTP formulations—reducing ROS production and DNA damage, likely due to improved Mn incorporation confirmed by EPR.

In parallel, Fe/TiO_2 NC biocompatibility was tested in NIH 3T3 and HS27 fibroblasts. Three formulations (NC-PP-50/400, -200/400, -500/400) were evaluated, revealing dose- and composition-dependent toxicity in NIH 3T3 cells, especially with higher Fe content. HS27 cells, however, showed minimal viability loss and low LDH release across all conditions. Microscopy indicated stronger surface interactions and aggregation in NIH 3T3 cultures. These results underscore the role of nanoparticle composition and cell type in determining biocompatibility, supporting the continued development of Mn-ZnO and Fe/TiO_2 nanomaterials for biomedical applications.

7.3. Results and discussion

7.3.1. Experiment 1 : MTS Assay Interference Test of NPs/NCs suspensions (Cell-Free Control)

Cell-free MTS assays confirmed minimal interference from both zinc oxide (ZnO) nanoparticles and iron oxide/titanium dioxide (Fe/TiO_2) nanocomposites across the tested concentration ranges. For ZnO and MnO NPs, slight absorbance increases were observed at concentrations ≥500 µg/mL—particularly for PVP-coated ZnO (ZnO15)—but all values remained close to baseline at concentrations ≤250 µg/mL, validating their compatibility with the MTS assay for cytotoxicity testing (Figure 59). Similarly, Fe/TiO_2 nanocomposites (CS, B, B2) suspended in either complete medium or HBSS showed no significant assay interference. Absorbance values were stable, with only minor variations noted in HBSS due to potential nanoparticle scattering, confirming their suitability for subsequent cell viability analyses (Figure 60).

7.3.2. Experiment 2 : Zinc Oxide Nanoparticles biocompatibility analysis

Cell Viability on 4 Cell Lines

Six ZnO NP types (undoped and Mn-doped at 500/5000 ppm; PVP or SHMTP coated) were tested on NIH 3T3, OK, CaCo-2, and MCF-7 cells. All showed dose-dependent cytotoxicity (Figure 5), with OK cells being the most resistant. Toxicity increased above 10 µg/mL, with no major differences between surfactants or doping levels, though SHMTP-based NPs showed sharper toxicity at ≥16 µg/mL.

NIH 3T3 Functional Tests

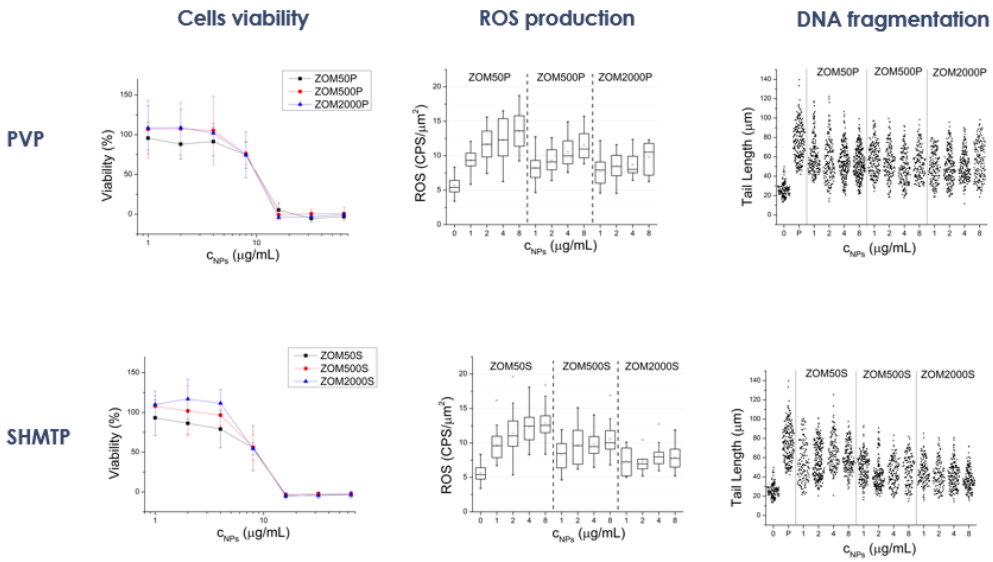

Figure 5 Cells viability ROS production and DNA fragmentation degree on NIH3T3 exposed with ZnO NPs [16]

At ≤4 µg/mL, cell viability remained high; it dropped to zero above 16 µg/mL. ROS production rose with concentration but was attenuated at higher Mn doping, especially in SHMTP samples. DNA damage followed a similar trend—higher Mn doping reduced fragmentation, most notably in ZOM2000S[16].

Conclusion

Higher Mn content in SHMTP-coated NPs reduced ROS and DNA damage, likely due to Mn's antioxidant activity. These findings support Mn-doped ZnO, particularly SHMTP-based, as more biocompatible at low doses.

7.3.3. Experiment 3: Fe/TiO2 Nanocomposite biocompatibility analysis

Three Fe/TiO_2 nanocomposites (NC-PP-50/400, NC-PP-200/400, NC-PP-500/400) were evaluated for cytotoxicity and membrane integrity in NIH 3T3 and HS27 fibroblasts(Figure 6). In NIH 3T3 cells, viability decreased with increasing Fe content, with NC-PP-500/400 reducing viability by ~30% at 150 µg/mL. In contrast, HS27 cells maintained >80% viability across all concentrations, indicating higher tolerance (Figure 6). LDH assays in HS27 confirmed minimal membrane damage, with only slight, non-significant increases at lower doses. These results suggest composition-dependent cytotoxicity in NIH 3T3 cells, likely linked to Fe-induced aggregation, while HS27 cells were more resistant, underscoring the importance of Fe/Ti ratio optimization and model selection in nanotoxicity assessments [21

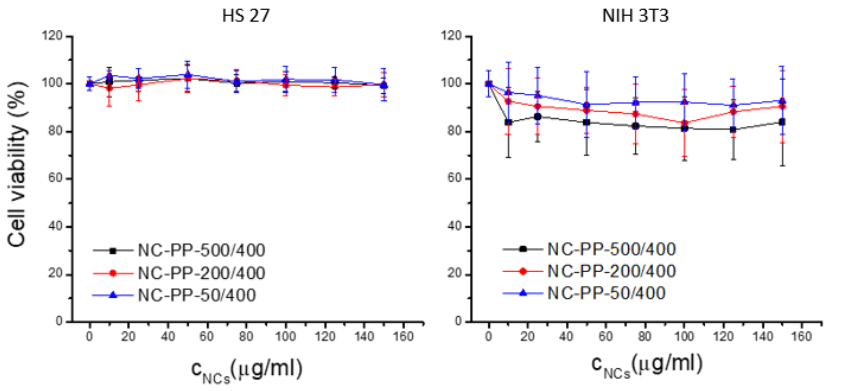

Figure 6 Cells viability test on HS27 and NIH3T3 exposec with iron oxide/TiO2 NC [21]

8. Conclusion and personal contributions

8.1. Conclusion

This doctoral research demonstrated the successful application of biophysical methods to assess platelet dysfunction in hematologic disorders and the cellular effects of engineered nanoparticles, offering valuable insight into disease mechanisms and nanomedicine safety.

Study 1 – Platelet Biophysics in Hematologic Disorders (Chapter 6):

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 (Chapter 7):

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.

Personal Contributions:

- Developed and optimized protocols for evaluating RMP, membrane fluidity, ROS, and platelet aggregation.
- Designed and executed all nanoparticle exposure experiments across murine and human cell models.
- Performed data analysis for viability, ROS, DNA damage, and LDH assays.
- Synthesized and validated findings into a framework connecting biophysical properties with therapeutic impact and safety in nanomedicine.

8.2. Perspectives:

This doctoral research optimized biophysical methods to assess platelet dysfunction and nanoparticle effects on malignant cells, establishing a platform for evaluating RMP, membrane fluidity, and oxidative stress. These tools show promises for improving diagnostics in MPNs and CLL. In nanomedicine, the study highlighted how Mn doping and surfactant choice can enhance ZnO and Fe/TiO_2 nanoparticle biocompatibility. Future work should explore these effects in advanced models and address key challenges like long-term safety and targeted delivery. Integrating biophysical assays with smart nanoparticle design offers a path toward safer, personalized therapies in oncology and thrombosis

9. Bibliography

1. Pasternak, C.A., *Transmembrane Channels and Disease*, in *Cell Function and Disease*, H. Eisenberg, Editor. 1989, Springer US: Boston, MA. p. 109-114.
2. Alberts, B., et al., *Molecular Biology of the Cell*. 6 ed. 2014, New York: Garland Science.
3. Boron, W.F. and E.L. Boulpaep, *Medical Physiology*. 3 ed. 2016, Philadelphia: Elsevier.
4. Winterbourn, C.C., A.J. Kettle, and M.B. Hampton, *Reactive oxygen species and antioxidants in inflammation: A review.* Free Radical Biology and Medicine, 2016. **40**(2): p. 215-257.
5. Lomakina, G.Y., Y.A. Modestova, and N.N. Ugarova, *Bioluminescence assay for cell viability.* Biochemistry (Moscow), 2015. **80**(6): p. 701-713.
6. Imamura, H., et al., *Approaches to monitor ATP levels in living cells: where do we stand?* FEBS Journal, 2021. **288**(11): p. 3602-3626.
7. Riss, T.L., et al., *Assay Guidance Manual*. 2004, Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences.
8. Michelson, A.D., *Platelets*. 4 ed. 2019, Cambridge, MA: Academic Press.
9. Olive, P.L. and J.P. Banáth, *The comet assay: a method to measure DNA damage in individual cells.* Nature Protocols, 2006. **1**(1): p. 23-29.
10. Bye, A.P., A.J. Unsworth, and J.M. Gibbins, *Platelet Shape Changes and Cytoskeleton Dynamics as Novel Therapeutic Targets for Anti-Thrombotic Drugs.* Journal of Thrombosis and Haemostasis, 2016
11. Wang, M., et al., *Inhibitors targeting Bruton's tyrosine kinase in cancers: drug development advances.* Leukemia, 2021. **35**: p. 312-326.
12. Papadakis, E., R. Hoffman, and B. Brenner, *Thrombohemorrhagic complications of myeloproliferative disorders.* Blood Rev, 2010. **24**(6): p. 227-32.
13. Gianazza, E., et al., *Platelets in Healthy and Disease States: From Biomarkers Discovery to Drug Targets Identification by Proteomics.* Int J Mol Sci, 2020. **21**(12).
14. Halkar, M. and A.M. Lincoff, *Dual antiplatelet therapy for acute coronary syndromes: How long to continue?* Cleve Clin J Med, 2016. **83**(9): p. 675-88.
15. Sukhanova, A., et al., *Dependence of nanoparticle toxicity on their physical and chemical properties.* Nanoscale research letters, 2018. **13**: p. 1-21.
16 Popescu, T., 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**(1): p. 18062.
17. Popov VM, Matei CO, Omer M, Onisai M, Matei MB, Savopol T, Bumbea H, Moisescu MG. Effects of Ibrutinib on biophysical parameters of platelet in patients with chronic lymphocytic leukaemia. Am J Blood Res. 2020 Dec 15;10(6):311-319.
18. Matei, C.O., et al., *Membrane Potential Evaluation of Platelets from Chronic Myeloproliferative Neoplasms and Thrombosis Patients.* Romanian Journal of Physics, 2025. **62**(9–10):
19 Talabieke, S., et al., *Arachidonic acid synergizes with aspirin preventing myocardial ischaemia–reperfusion injury and mitigates bleeding risk.* Cardiovascular Research, 2025. **121**(5): p. 775-787.
20 Zhang, Y., et al., *Biomedical Applications of Zinc Oxide Nanomaterials.* Current Molecular Medicine, 2013. **13**(10): p. 1633-1645.
21 Popescu, T., et al., *Facile synthesis of low toxicity iron oxide/TiO(2) nanocomposites with hyperthermic and photo-oxidation properties.* Sci Rep, 2022. **12**(1): p. 6887.

Published articles:

- **Ibrutinib on biophysical parameters of platelet in patients with chronic lymphocytic leukaemia**
  Popov, Viola & **Matei, Christien Oktaviani** & Omer, Meilin & Matei, Mircea & Savopol, Tudor & Bumbea, Horia & Moisescu, Mihaela. (2020). Effects of. American journal of blood research. 10. 311-319.
- *(This article is covered in chapter 6.3.2 CLL patients without thrombosis)*

- **Influence of surfactant-tailored Mn-doped ZnO nanoparticles on ROS production and DNA damage induced in murine fibroblast cells**
  Popescu, T., **Matei, C.O.,** Vlaicu, I.D. *et al.* Influence of surfactant-tailored Mn-doped ZnO nanoparticles on ROS production and DNA damage induced in murine fibroblast cells. Scientific Reports 10, 18062 (2020). https://doi.org/10.1038/s41598-020-74816-0
- *This article is covered in chapter 7.3.2. Experiment 2 : Zinc Oxide Nanoparticles biocompatibility analysis)*

- **Facile synthesis of low toxicity iron oxide/TiO2 nanocomposites with hyperthermic and photo-oxidation properties.**
  Popescu, Traian & **Matei, Christien Oktaviani** & Culita, Daniela & Maraloiu, Valentin-Adrian & Rostas, Arpad & Diamandescu, L. & Iacob, Nicusor & Savopol, Tudor & Ilas, Monica & Feder, Maik & Lupu, Andreea & Iacoban, Alexandra & Vlaicu, Ioana & Moisescu, Mihaela. (2022). Scientific Reports. 12. 10.1038/s41598-022-11003-3.
- *This article is covered in chapter 7.3.3. Experiment 3: Fe/TiO2 Nanocomposite biocompatibility analysis)*

- **Membrane potential evaluation of platelets from chronic myeloproliferative neoplasms and thrombosis patients**
  C.O. Matei, V.M. Popov, M.B. Matei, E. Kovacs, M.G. Moisescu, T. Savopol. (2025). Romanian Journal of Physics

  (This article is covered in chapter 6.3.3. MPNs patients with and without thrombosis)