CELL TECHNOLOGIES - RESEARCH TOPICS

SOFIA UNIVERSITY - FACULTY OF BIOLOGY

- LIVE-CELL BIOSENSORS FOR BIOCOMPATIBILITY ASSESSMENT

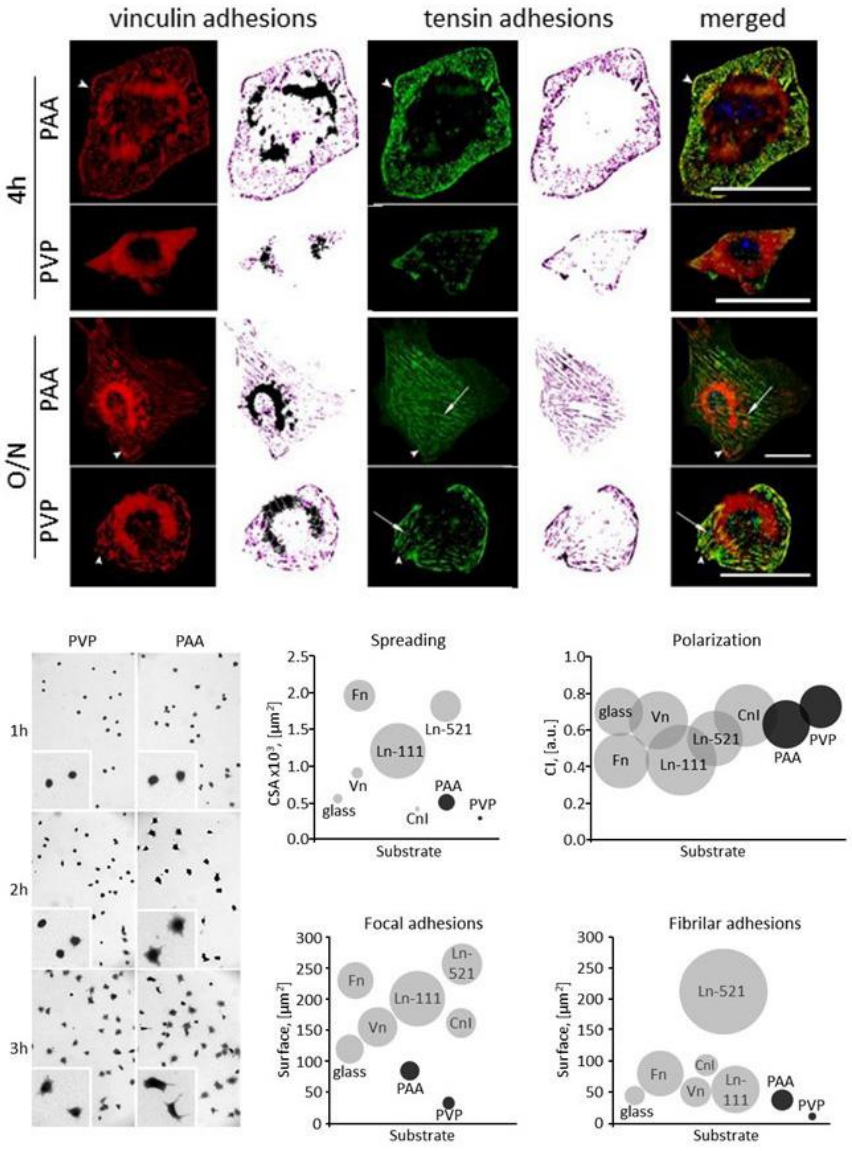


Fig 1. Assessment of surface biocompatibility of poly(dimethylsiloxane-b-acrylic acid) (PAA) - and poly(dimethylsiloxane-b-vinyl pyrrolidone) (PVP) – based substrates using live-cell biosensor.

One of the most advanced and emerging trends in the area of biosensors is the use of live cells for collection, processing and interpretation of environmental signals. Their advantage lies in the ability to perform a simultaneous assessment of a complex combination of environmental parameters influencing biological systems such as adhesion, presence of specific signals regulating cell differentiation, physical and chemical characteristics of the substrate, migration, cytotoxicity, etc.

The generation of genetically manipulated cells with properties of biosensors is widely used in assessing the quality of new materials created for the needs of regenerative medicine and developing new approaches to differentiation of stem cells.

Activities and scientific expertise:

1. Establishment of cell lines stably expressing fluorescent-labeled proteins of the adhesome complex – biosensors for assessment of surface biocompatibility

2. Standardization of the behavior of biosensors (spreading, polarization, development of focal and fibrillary adhesions, migration, etc.) on natural biological substrates биологични

3. Use of biosensors to assess the surface biocompatibility of new materials developed for the needs of regenerative medicine

Technical capacity:

- Establishment of lines / clones of eukaryotic cells stably expressing exogenous, fluorescent-labeled proteins

- Characterization of behavior (expression levels, post-translational modifications, cell localization) of exogenous fusion proteins stably expressed in eukaryotic cells

- Assessment of the adhesive potential of natural biomaterials and artificial polymeric substrates

Team:

Prof. Roumen Pankov, Assist. Prof. Nadezhda Stefanova; Assist. Prog. Georgi Georgiev; PhD and M.Sc. students

Partners:

Institute of biophysics and biomedical engineering (IBBE) – Bulgarian Academy of Sciences

Relevant publications:

- Ivanova SI, Chakarov S, Momchilova A and Pankov R. Live-cell biosensor for assessment of adhesion qualities of biomaterials. *Materials Science and Engineering: C*. 78: 230-238, 2017.
- Svetlana I.Ivanova,Stoyan A.Chakarov,Roumen G.Pankov Formation of Fibrillar Adhesions Correlates with Spreading but Does not Depend on Cell Polarization *Comptes rendus de l'Acade'mie bulgare des Sciences* 71 (11): 1495-1501, 2018
- Pankov R and Momchilova A., Cell Adhesions and Signaling – a Tool for Biocompatibility Assessment, In: NATO Science for Peace and Security Series A: Chemistry and Biology, p. 1-20, Springer Verlag, Edd. Shastri V., and Altankov, G., 2010
- Green JA, Berrier AL, Pankov R, Yamada KM., Beta1 integrin cytoplasmic domain residues selectively modulate fibronectin matrix assembly and cell spreading through talin and Akt-1. J Biol Chem. 284(12):8148-59, 2009.

- Pankov R and Momchilova A., Fluorescent labeling techniques for investigation of fibronectin fibrillogenesis, Sharona Even-Ram and Vira Artym (eds.), *Methods Mol Biol*.522:261-74, Humana Press, 2009.
- Roumen Pankov, Yukinori Endo, Sharona Even-Ram, Masaru Araki, Katherine Clark Edna Cukierman, Kazue Matsumoto, and Kenneth M. Yamada, A Rac Switch Regulates Random versus Directionally Persistent Cell Migration, *J Cell Biol*. 170: 793-802, 2005. 46.
- Clark K, Pankov, R, Travis MA, Askari JA, Mould AP, Craig SE, Newham P, Yamada KM, Humphries MJ. A specific α5β1-integrin conformation promotes directional integrin translocation and fibronectin matrix formation. *J Cell Sci.* Jan 15;118(Pt 2):291-300, 2005.
- Pankov, R., Cukierman, E., Katz, B-Z., Matsumoto, K., Lin, D.C., Lin, S., Hahn, C., and Yamada, K.M. Integrin dynamics and matrix assembly: tensin-dependent translocation of $\alpha_5\beta_1$ integrins promotes early fibronectin fibrillogenesis. *J. Cell Biol.* 148: 1075-1090, 2000.

Projects:

- Cell adhesive contacts – potential sensory element for assessment of surface biocompatibility, Contract ДО1-1261/07 – leader – Prof. R. Pankov

- DERIVATION AND DIFFERENTIATION OF PLURIPOTENT AND MULTIPOTENT STEM CELL LINES

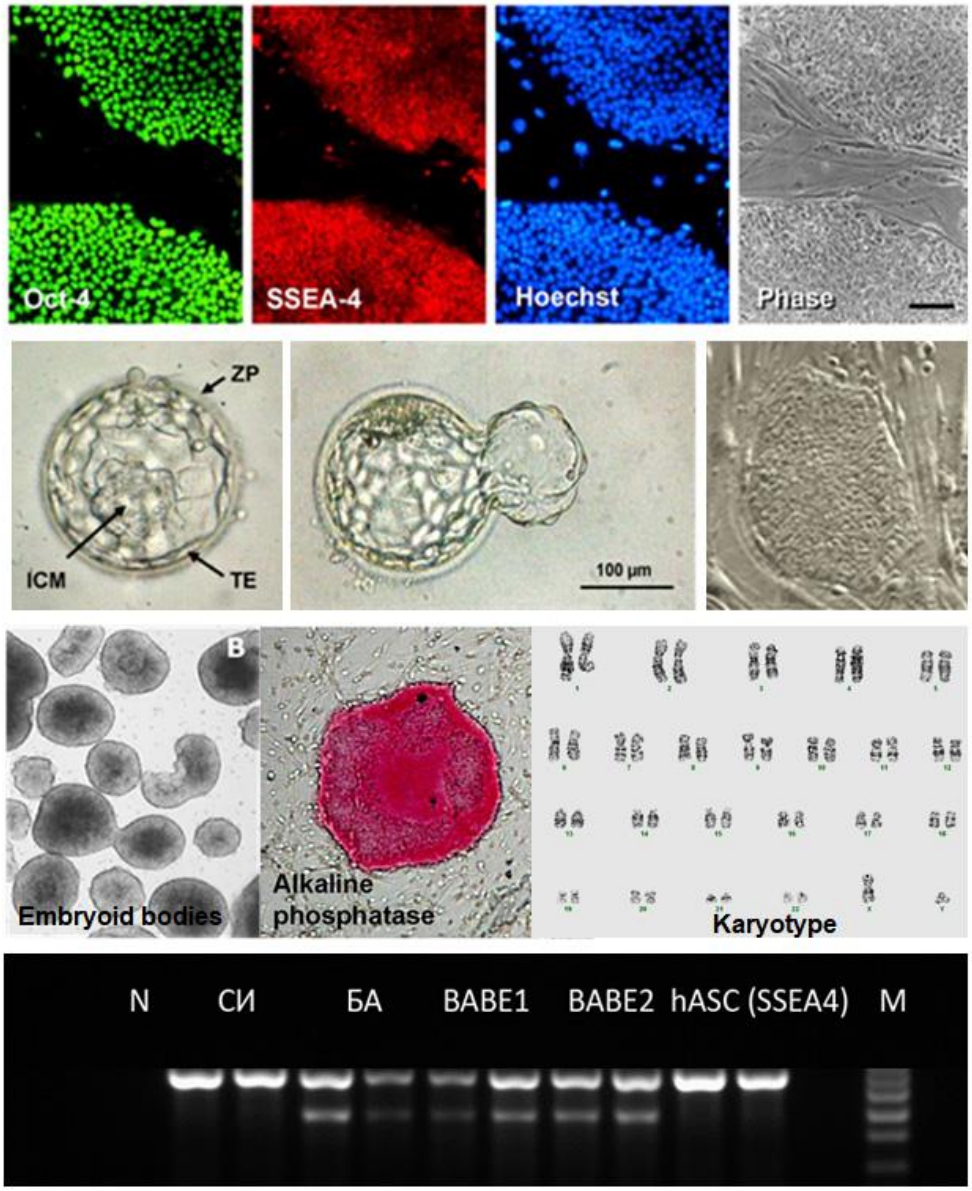


Fig. 2 Characterization of hESC line

Stem cells are characterized by their significant capacity for self-renewal and differentiation into different cell types of the adult organism. This provides the opportunity for laboratory production of a large number of differentiated cells of normal karyotype suitable for research and high-efficiency testing of new pharmacological agents. The aim of our group is to establish a set of well-characterized lines of pluripotent and multipotent stem cells from humans and different animal species as well as to introduce existing and / or optimized protocols for their differentiation.

Activities and scientific expertise:

1. Establishment of human embryonic and mesenchymal stem cell lines

2. Characterization of stem cells based on expression of specific markers (immunofluorescence, immunoblotting, embryoid bodies, etc.)

3. Introduction, optimization and verification of new protocols for stem cell differentiation

Technical capacity:

- Generation of human embryonic stem cell lines (isolation of internal cell mass, cultivation on a feeder layer, cryopreservation)

- Characterization of pluripotent and multipotent stem cell (immunofluorescence-specific expression of markers, PCR, embryoid body formation, karyotyping)

- Stem cell differentiation (neural, epithelial, mesenchyme cell cultures)

Team:

Prof. Rumen Pankov; Assist. Prof. Borislav Arabadjiev; Assist. Prof. Petar Eftimov, PhD and M.Sc. students

Partners:

Institute of biology and immunology of reproduction (IBIR) – Bulgarian Academy of Sciences

Relevant publications:

- Arabadjiev B, Petkova R, Chakarov S, Pankov R, Zhelev N. We heart cultured hearts. A comparative review of methodologies for targeted differentiation and maintenance of cardiomyocytes derived from pluripotent and multipotent stem cells. BioDiscovery 2014; 14: 2; DOI: 10.7750/BioDiscovery.2014.14.2
- Petkova, R., Arabadjiev, B., Chakarov, S., & Pankov, R. Current state of the opportunities for derivation of germ-like cells from pluripotent stem cells: are you a man, or a mouse?. Biotechnology & Biotechnological Equipment, 28(2), 184-191, 2014.
- Петкова Р., Чакъров С., Панков Р., Стволови клетки, 2012, Академично издателство „Проф. Марин Дринов" ISBN 978 954 322 517 0, второ издание 2014 год., ISBN 978-954-322-798-3
- Arabadjiev B, Petkova R, Momchilova A, Chakarov S, Pankov R. Of mice and men – differential mechanisms of maintaining the undifferentiated state in mESC and hESC. Biodiscovery 2012; 3: 1; DOI: 10.7750/BioDiscovery.2012.3.1
- Arabadjiev B, Petkova R, Nonchev S, Chakarov S, Momchilova A, Pankov R, Derivation of human embryonic stem cell line from discarded ivf morula, Comptes Rendus de L'Academie Bulgare des Sciences, 63 (12), 1765-1770, 2010
- Arabadjiev B, Petkova R, Chakarov S, Momchilova, A and Pankov R., Do we need more human embryonic stem cell lines?, Biotechnology & Biotechnological Equipment, 24(3),1921-1927, 2010..

Projects:

- Generation of embryonic stem cell lines and assessment of their capacity to differentiate to oocytes in vitro, МОН, ДО 02-180/08– leader – prof. R. Pankov

- THREE-DIMENSIONAL (3D) CELL CULTURES

The current knowledge of cellular behavior has been accumulated mainly from studies on cells cultured as conventional monolayer cultures. This simplified approach has provided a vast amount of knowledge, but current data show that growing cells on artificial rigid and flat plastic substrates leads to substantial differences in basic physiological processes such as adhesion, proliferation, cell signaling, migration and apoptosis compared to the elastic three-dimensional tissue environment. This requires the development of three-dimensional (3D) cell cultures that successfully mimic in vivo conditions in the multicellular organism. The development of new 3D culture methods provides the opportunity to obtain in vivo relevant knowledge as well as to evaluate the impact of different biologically active substances in in vivo-like conditions.

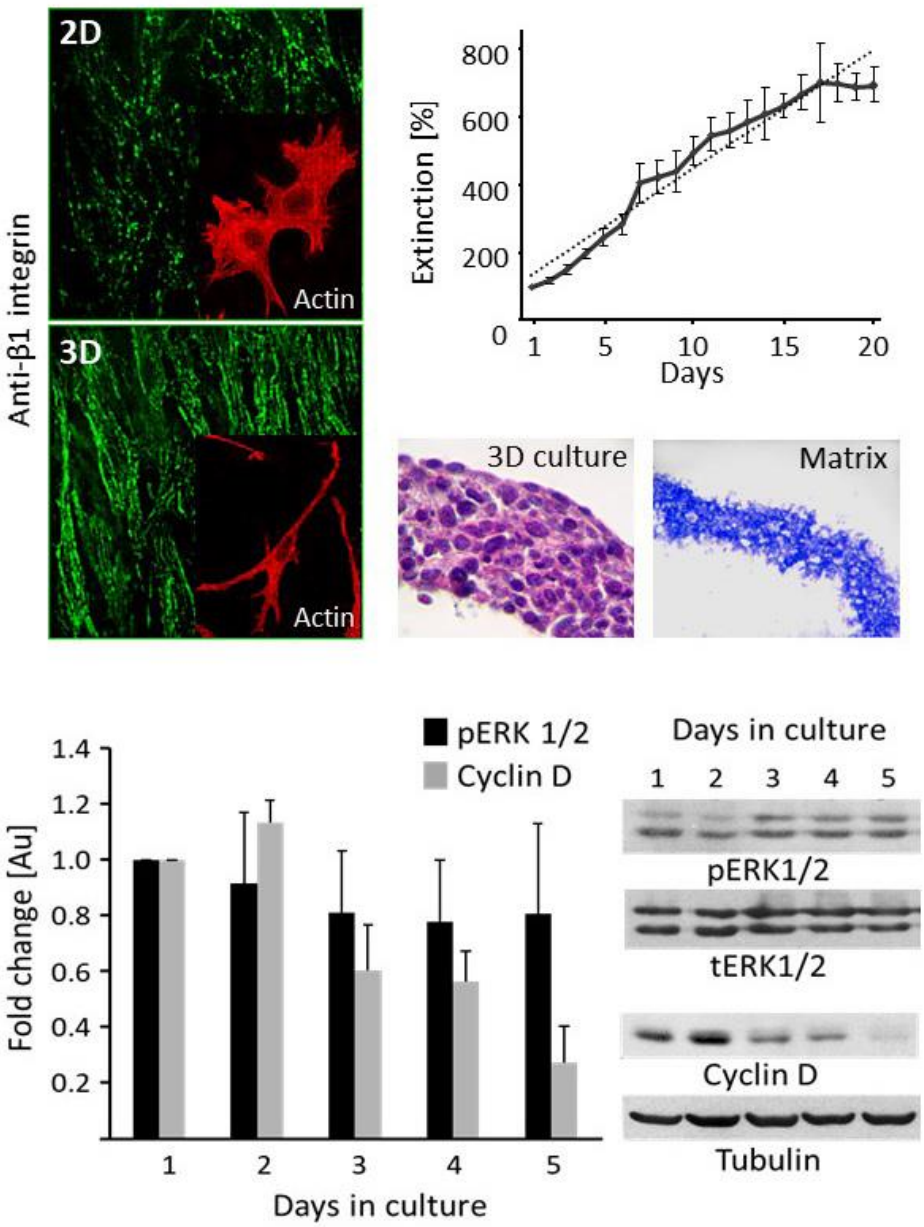


Fig. 3. Characterization of 3D cell culture of GD25 fibroblast cell line.

Activities and scientific expertise:

1. Development of 3D cell cultures based on cell-produced, natural extracellular matrix

2. Characterization of cell signaling regulating proliferation, differentiation, migration activity and apoptosis in 3D cell cultures

3. Examination of mechanisms of impact of biologically active substances in in vivo-like conditions using 3D cell cultures

Technical capacity:

- Development of 3D cell cultures based on cell-accumulated, natural extracellular matrix

- Assessment of changes in cell morphology, signaling and physiology (immunofluorescence, immunoblotting with phosphor-specific antibodies, proliferations, apoptosis, migration, aging tests, etc.)

Team:

- Prof. Roumen Pankov, Assist. Prof. Nadezhda Stefanova; Assist. Prog. Georgi Georgiev; PhD and M.Sc. students

Partners:

Institute of biophysics and biomedical engineering (IBBE) – Bulgarian Academy of Sciences

Relevant publications:

- Skrobanska, R., Evangelatov, A., Stefanova, N., Topouzova-Hristova, T., Momchilova, A., & Pankov, R. Cell proliferation in in vivo-like three-dimensional cell culture is regulated by sequestration of ERK1/2 to lipid rafts. Cell proliferation.47(4), 336-346, 2014
- Aleksandar Evangelatov and Roumen Pankov The Evolution of Three-Dimensional Cell Cultures Towards Unimpeded Regenerative Medicine and Tissue Engineering, Regenerative Medicine and Tissue Engineering, Prof. Jose A. Andrades (Ed.), (2013). ISBN: 978-953-51-1108-5, InTech, DOI: 10.5772/55564. Available from: http://www.intechopen.com/books/regenerative-medicine-and-tissue-engineering/the-evolution-of-three-dimensional-cell-cultures-towards-unimpeded-regenerative-medicine-and-tissue-
- Staneva G, Lupanova T, Chachaty C, Petkova D, Koumanov K, Pankov R, Momchilova A. Structural organization of plasma membrane lipids isolated from cells cultured as a monolayer and in tissue-like conditions. J Colloid Interface Sci. Jul 1;359(1):202-9, 2011
- Alexander Evangelatov, Ralica Skrobanska, Alexander Kyumurkov, Roumen Pankov, Three-Dimensional Environment Stimulates RhoA Activation in Fibroblast Cells, Comptes Rendus de L'Academie Bulgare des Sciences, 64(4), 553-558, 2011
- Lupanova T, Stefanova N, Petkova D, Staneva G, Jordanova A, Koumanov K, Pankov R, Momchilova A., Alterations in the content and physiological role of sphingomyelin in plasma membranes of cells cultured in three-dimensional matrix, Mol Cell Biochem., 340 (1-2), 215-222, 2010.
- Stefanova N, Staneva G, Petkova D, Lupanova T, Pankov R, Momchilova A. Cell culturing in a three-dimensional matrix affects the localization and properties of plasma membrane cholesterol, Cell Biol Int. Oct;33(10):1079-86, 2009.

- Jordanova A, Stefanova N, Staneva G, Pankov R, Momchilova A, Lalchev Z. Surface properties and behavior of lipid extracts from plasma membranes of cells cultured as monolayer and in tissue-like conditions, Cell Biochem Biophys. 54(1-3):47-55, 2009.
- Damianova R, Stefanova N, Cukierman E and Momchilova A, Pankov R, Three-dimensional matrix induces sustained activation of ERK1/2 via Src/Ras/Raf signaling pathway, Cell Biol International, 32:229-34, 2008.
- Cukierman, E., Pankov, R., and Yamada, K. M. Cell interactions with 3D matrices. Curr. Opin. Cell Biol. 14: 633-640, 2002.
- Cukierman, E., Pankov, R., Stevens, D. R., and Yamada, K. M. Taking cell-matrix adhesions to the third dimension. Science 294: 1708-1712, 2001.

Projects:

- Cell quiescence: Role of cell compartmentalization of extracellular signal-regulated kinases (ERK), FNI, ДФНИ-Б02/13/14– leader – Prof. R. Pankov
- Transmembrane transport of signals in 3D matrix, MON, Contract ВУ-Б-01/05 – leader - Prof. R. Pankov
- Examination of the ultrastructure organization of 3D cell culture of GD25 mouse fibroblasts , SU «St. Kliment Ohridski», Contract № 088/2007– leader - Prof. R. Pankov

- NEW NANOPARTICLES WITH POTENTIAL FOR APPLICATION IN BIOMEDICINE - BIOLOGICAL ACTIVITY, INTERNALIZATION AND DELIVERY OF BIOACTIVE COMPOUNDS

Due to the increased scientific interest in nanoparticles as transporters biologically active substances, our working group investigates the cytotoxicity, internalization and release of the biologically active load of new nanoparticles, polyplexes or nanocapsules created by the group at the Institute of Polymers, BAS. Nano-carriers are designed to be biocompatible, non-toxic and biodegradable. Internalization and load release can be accomplished through different pathways in the cell - endocytosis (clathrin or caveolin-dependent), enzymatic, lysosomal secretion, vesicular secretion, and controlled diffusion or gradual breakdown of the polymeric shell. Different mechanisms are important for the efficiency of nanomaterials in biomedical research and can be tracked by direct (microscopic) and indirect methods.

Activities and scientific expertise:

1. Assessment of cytotoxicity (direct and in result of potential metabolization) through enzyme-based quantitative tests;

2. Assessment of morphological alterations; membrane permeability, cell proliferation, etc. Examination of internalization routes – endocytosis or transmembrane;

3. Transfection efficiency tests (for particles carrying DNA)

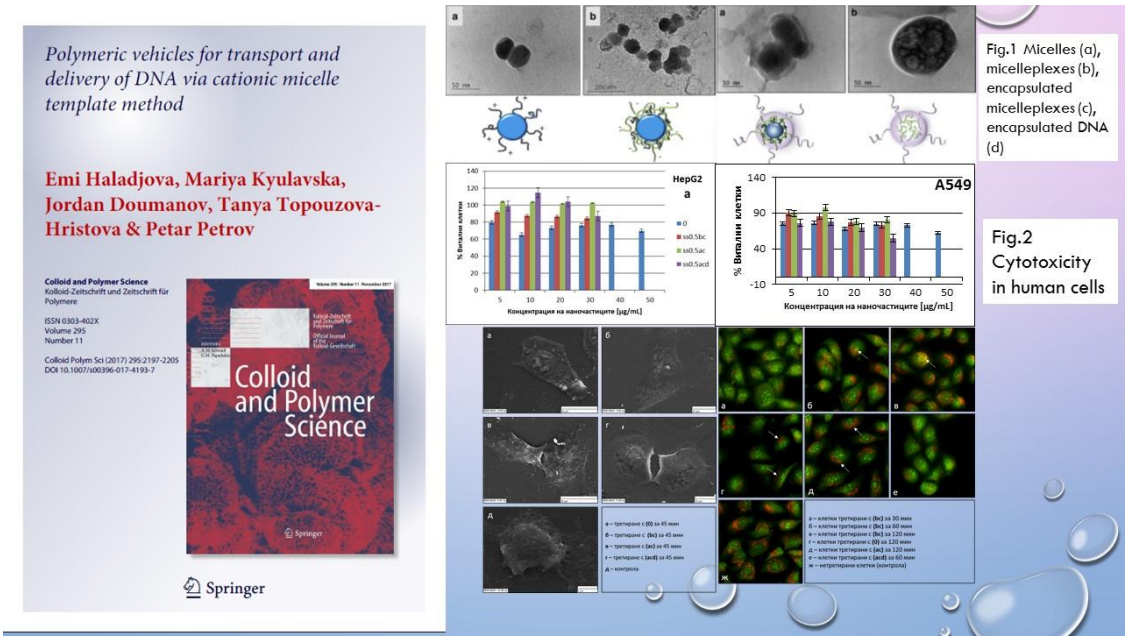


Fig. 4 Polymeric vehicles for DNA delivery

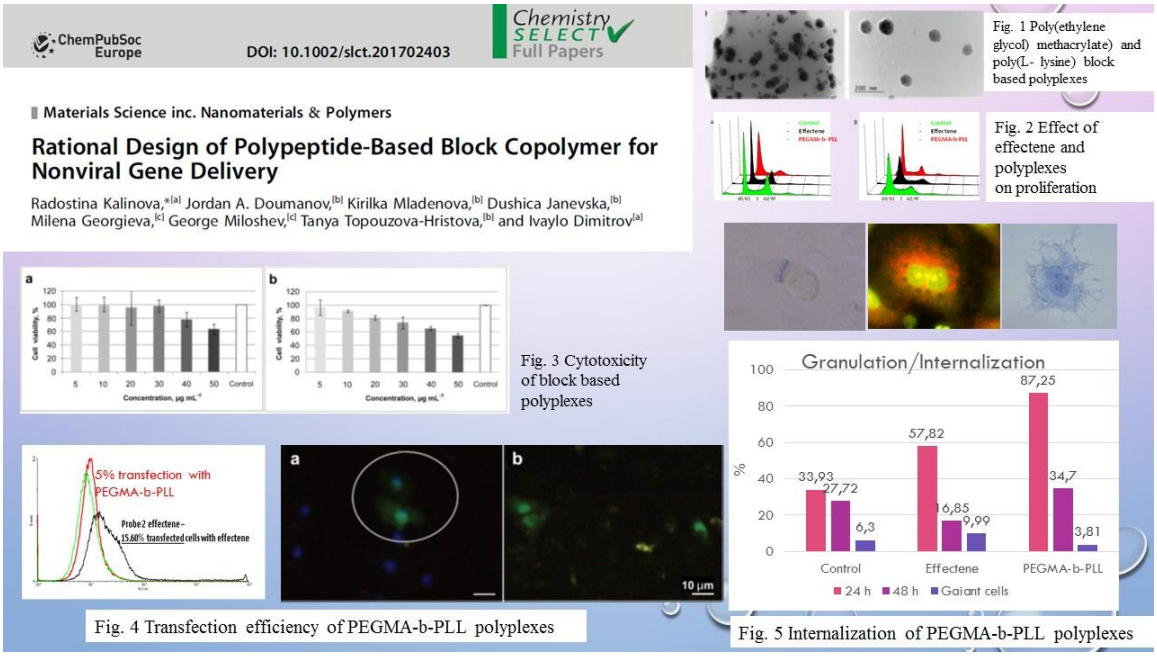


Fig. 5 Nanoparticles internalization in human cells

Technical capacity:

- Cell cultures, cell manipulations (transfection, immunofluorescent labeling, etc.), biochemical and microscopy tests (light, phase-contrast, fluorescent, confocal, TEM and SEM), image analysis, cytotoxicity tests (MTT, MTC, comet assay, AnexinV / PI, Cytodeath, etc.).

Team:

Prof. Svetla Petrova, Assoc. Prof. Tanya Topuzova-Hristova, Assoc. Prof. Vesselina Moskova-Dumanova, Assoc. Prof. Yordan Dumanov, Assist. Prof. Kirilka Mladenova, Ass. Pavel Vedev, Ph.D. student Ralitza Veleva, lab technician Denitsa Melnishka

Partners: Institute of polymers, BAS

Relevant publications:

- E. Haladjova, S. Halacheva, D. Momekova, V. Moskova-Doumanova, T. Topouzova-Hristova, K. Mladenova, J. Doumanov, M. Petrova, S. Rangelov. Polyplex Particles Based on Comb-Like Polyethylenimine/Poly(2-ethyl-2-oxazoline) Copolymers: Relating Biological Performance with Morphology and Structure. Macromol. Biosci. 2018, 1700349. https://doi.org/10.1002/mabi.201700349
- Radostina Kalinova, Jordan A. Doumanov, Kirilka Mladenova, Dushica Janevska, Milena Georgieva, George Miloshev, Tanya Topouzova-Hristova, and Ivaylo Dimitrov. Rational Design of Polypeptide-Based Block Copolymer for Nonviral Gene Delivery, Chemistry Select 2017, 2, 12006 – 12013; DOI: 10.1002/slct.201702403
- Haladjova, E., Kyulavska, M., Doumanov, J., Topouzova-Hristova, T., Petrov, P. Polymeric vehicles for transport and delivery of DNA via cationic micelle template method. Colloid Polym Sci (2017). https://doi.org/10.1007/s00396-017-4193-7

- T. Vladkova, I. Ivanova, A. Staneva, M. Albu-Kaya, A. Shalaby, V. Moskova-Doumanova, A. Kostadinova, Preparation and Biological Activity of New Collagen Composites, Part III. Collagen/(Ag/RGO) and Collagen/(Ag/RGO/SiO 2) Composites. Journal of Archives in Military Medicine, 5(2), 2017, e57454
- Todorka G. Vladkova, Iliana A. Ivanova, Anna D Staneva, Madalina G Albu, Ahmed S A Shalaby, Tanya I Topouzova, Anelia S Kostadinova. Preparation and Biological Activity of New Collagen Composites Part II: Collagen/Reduced Graphene Oxide Composites. J Arch Mil Med. 2017 February; 5(1):e46406; doi: 10.5812/jamm.46406
- Madalina G. Albu, Todorka G. Vladkova , Iliana A. Ivanova, Ahmed S. A. Shalaby, Veselina S. Moskova-Doumanova, Anna D. Staneva, Yanko B. Dimitriev, Anelya S. Kostadinova, Tanya I. Topouzova-Hristova. 2016. Preparation and Biological Activity of New Collagen Composites, Part I: Collagen/Zinc Titanate Nanocomposites. Applied Biochemistry and Biotechnology, 180(1):177-93; DOI 10.1007/s12010-016-2092-x
- Emi Radoslavova Haladjova, Silvia S Halacheva, Vilma Posheva, Ekaterina Peycheva, Veselina Moskova-Doumanova, Tanya Topouzova-Hristova, Jordan Doumanov, Stanislav Miletiev Rangelov. Comb-like Polyethyleneimine-based Polyplexes: Balancing Toxicity, Cell Internalization, and Transfection Efficiency via Polymer Chain Topology. 2015. Langmuir 31 (36), pp 10017–10025 DOI:10.1021/acs.langmuir.5b02408
- T. Topouzova-Hristova, K. Mladenova, V. Moskova-Doumanova, R. Kalinova, E. Haladjova, I. Dimitrov, S. Rangelov, J. Doumanov. 2015. METHOD FOR DETECTION OF POLYCATIONIC NANOPARTICLES LOADED WITH DNA IN EUKARYOTIC CELLS, Science & Technologies, том:5, брой:3, стр.87-91

Projects:

- Probiotics and health: mechanisms of effectiveness in selected Bulgarian strains of lactic acid bacteria MES, КП-06-ОПР03 / 16;
- Association of gastrointestinal-1 membrane domains in model monolayers and epithelial cells - prerequisite for innovative therapies for retinal degeneration, MES, КР-06-, Head: Assoc. Prof. Yordan Dumanov
- Design of new supramolecular nanoparticles: spherical nucleic acids with polymeric and liposomic nuclei, MES, DN 19/8
- Phytochemical and biological study of Inula (Asteraceae) species from Bulgarian flora - new sources of biologically active substances, Ministry of Education and Science, No. 09/11 of 16.12.2016
- New polymers, polymer nanoparticles and nanocapsules for transport of biological molecules, MES, DFSI-T02 / 7

- CELL SIGNALING, PROTEIN SORTING AND CELL POLARIZATION
- MOLECULAR-BIOLOGY STDUIES ON SECRETORY PHOSPHOLIPASES A2 UNDER PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL CONDITIONS

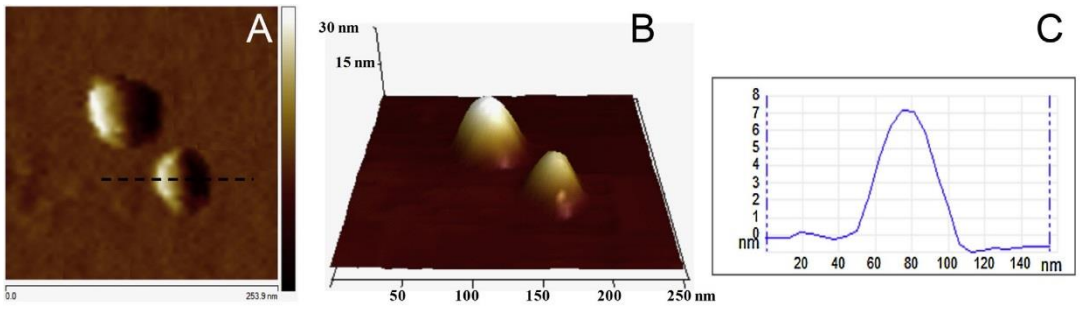
A basic model used in the cell polarization and protein sorting studies in our group is human bestrophin-1 (hBest1) a transmembrane protein, predominantly expressed in the basolateral membranes of retinal pigment epithelium (RPE) and neuronal cells, a member of Ca2+-dependent anion channel family with still unclear function. Mutations in the BEST1 gene, coding protein hBest1 in the retinal pigment epithelium, are responsible for generalized damages of the retina, known as bestrophinopathies. Its participation in the pathology of Alzheimer's, Parkinson's and other brain diseases (stroke, epilepsy, etc.) has been determined. Using model membrane systems, we have shown the secondary structure of purified hBest1 and that interactions between pure hBest1 and POPC monolayers occur in the polar head regions of the phospholipids. Our research is aimed to provide an up-to-date model for the molecular mechanism of hBest1 activity.

The secretory PLA2s (EC 3.1.1.4) play a crucial role in a variety of physiological processes - remodeling of phospholipid membranes, prostaglandin biosynthesis, cell proliferation and signal transduction. Some of them contain specific structural “pharmacological” site, other than the enzyme active center which is responsible for their pharmacological effects (neurotoxicity, myotoxicity, cardiotoxicity, anticoagulation/coagulation, platelet aggregation, hemolytic effect, tissue damage, cytokine and chemokine production, inflammatory processes) with not well understood molecular mechanisms. This requires design and targeted synthesis of specific inhibitors enabling a selective control of enzyme and pharmacological activities in order to elucidate the molecular mechanisms of toxicity and eventual application in clinical diagnostics.

Activity and research expertise:

Investigation of pharmacological effects - maintenance of membrane homeostasis, cyto- and neurotoxicity, apoptosis, induction of inflammatory processes, cell signaling in cancerous and non-cancerous cells, potential involvement in neurodegenerative diseases. Biomolecular interactions analysis (protein-protein, protein-lipid).

Cell culture. Isolation, purification and characterization of proteins, nucleic acids, lipids and sugars. Enzymatic kinetics and pharmacokinetics. Inhibition of enzymatic and pharmacological activities. Model membrane systems - Langmuir monolayers, liposomes. Tensiometric investigations. A potential model for diagnostic purposes.



Фиг. 6 Representative tapping mode AFM images of hBest1 LB-films on glass slides transferred at surface pressure 20 mN/m in (A) error mode and (B) 3D mode. (C) Heightprofile from the dashed line in (A).

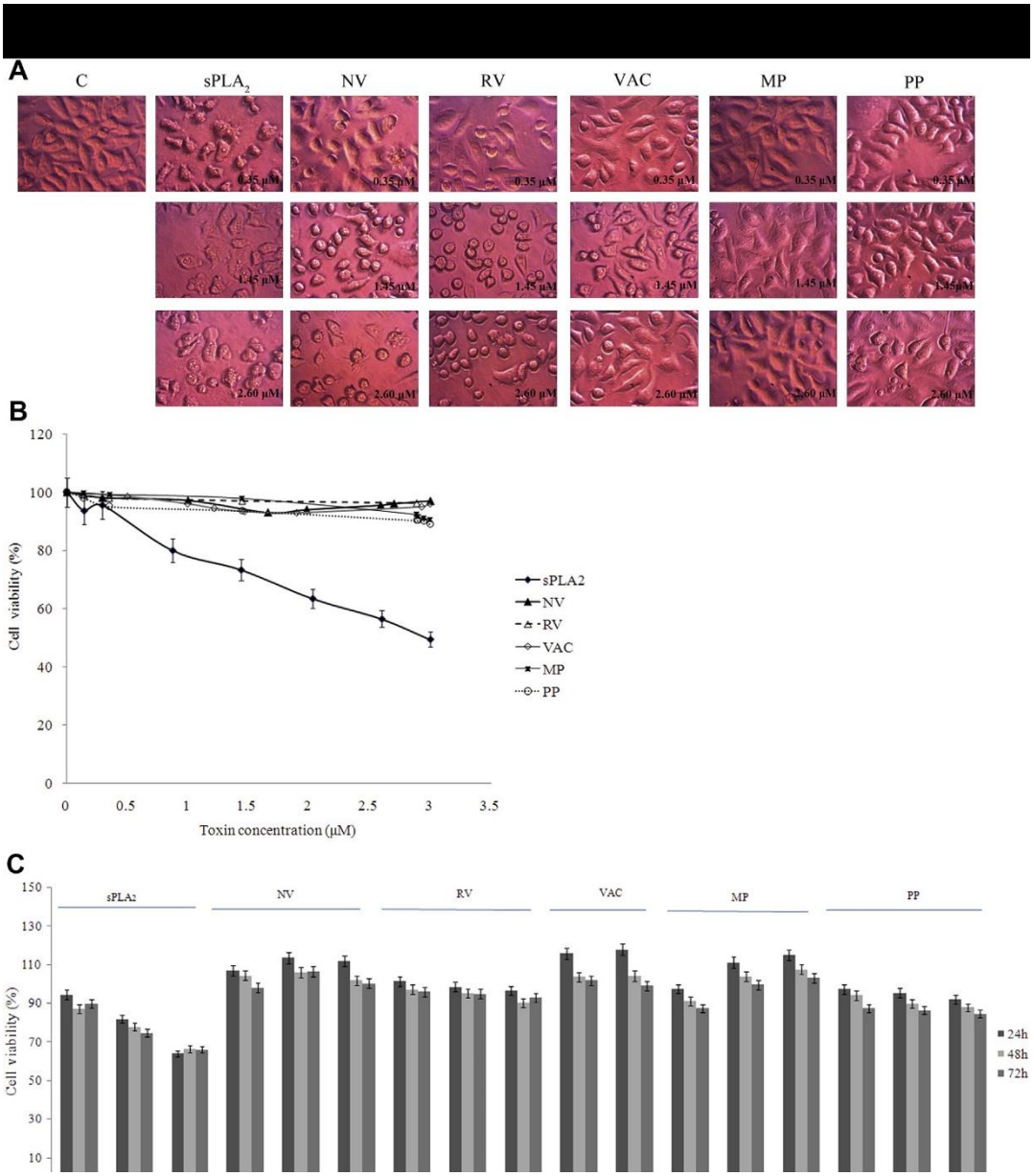


Fig. 7 A. Light microscopy images of HepG2 cells treated with: sPLA2; NV; RV; VAC; MP; and PP, made after 2 h of incubation. B. Cell viability of HepG2 cells (2 x 10^5), following 2 h treatment with different concentrations of: $sPLA_2$; NV; RV; VAC; MP; and PP. Data are represented as means ± SEM (n = 3). C. Cell viability of HepG2 cells (2x 105) assayed at 24 h, 48 h and 72 h of cultivation in a new medium at 37 °C, after treatment of the cells for 2 h with different concentrations of: $sPLA_2$; NV; RV; VAC; MP; and PP. Data are represented as
means ± SEM (n = 3).

Technological capacity

Chromatographic, spectrophotometric and electrophoretic methods of proteins and nucleic acids (2D electrophoresis, proteomics), Western blot, carbohydrates analyses, different enzyme activity assays, enzyme kinetics, enzyme inhibition, model membrane methods, tensiometric methods.

Research group

Prof. Dr. Svetla Petrova, Assoc. Prof. Yordan Dumanov, PhD. Assoc. Porf. Dr. Tanya Topuzova-Hristova, Assoc. Prof. Dr. Vesselina Svetoslavova Moskova-Dumanova, PhD students, post-doctoral students

List of most relevant publications

Jordan Doumanov , Kirilka Mladenova , Tanya Topouzova-Hristova , Stoyanka Stoitsova, Svetla Petrova , Effects of vipoxin and its components on HepG2 cells, Toxicon 94 (2015) 36-44, IF (2,581)

Jordan Doumanov, Kirilka Mladenova, Radoslav Aleksandrov, Georgi Danovski, Svetla Petrova, Investigation of pharmacologically active snake venom sPLA2 with different cell lines, Biotechnology and biotechnological equipment, 2014, http://dx.doi.org/10.1080/131028a 18.2014.965014(IF 0.622)

Doumanov JA, Zeitz C, Dominguez Gimenez P, Audo I, Krishna A, Alfano G, Diaz ML, Moskova-Doumanova V, Lancelot ME, Sahel JA, Nandrot EF, Bhattacharya SS., Disease-causing mutations in BEST1 gene are associated with altered sorting of bestrophin-1 protein, Int J Mol Sci., 2013 Jul 22;14(7):15121-40. doi: 10.3390/ijms140715121, (IF 2.464)

Kirilka Mladenova, Svetla D. Petrova, Georgi As. Georgiev, Veselina Moskova-Doumanova, Zdravko Lalchev, Jordan A. Doumanov, Interaction of Bestrophin-1 with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in surface films, Colloids and Surfaces B: Biointerfaces, 2014, doi: 10.1016/j.colsurfb.2014.01.045, in press, (IF 4.287)

Kirilka Mladenova, Svetla D. Petrova, Tonya D. Andreeva, Veselina Moskova-Doumanova, Tanya Topouzova-Hristova, Yuri Kalvachev, Konstantin Balashev, Shomi S. Bhattacharya, Christina Chakarova, Zdravko Lalchev, Jordan A. Doumanov, Effects of Ca2+ ions on bestrophin-1 surface films, Colloids and Surfaces B: Biointerfaces,2017, 149 (2017) 226–232, http://dx.doi.org/10.1016/j.colsurfb.2016.10.023 (IF 3.902)

Tonya D. Andreeva, Svetla D. Petrova, Kirilka Mladenova, Veselina Moskova-Doumanova, Tanya Topouzova-Hristova, Yulia Petseva, Nikola Mladenov, Konstantin Balashev, Zdravko Lalchev, Jordan A. Doumanov, Effects of Ca2+, Glu and GABA on hBest1 and composite hBest1/POPC surface films, Colloids and Surfaces B: Biointerfaces 161 (2018) 192–199(IF 4.295)

Projects

1. Association of bestrophin-1 with membrane domains in model monolayers and epithelial cells - a prerequisite for innovative therapies for retinal degenerations, КР-06-Н23/7 от 18.12. 2018, Bulgarian Science Fund, coordinator Assoc. Prof. Dr. Jordan Doumanov
2. Comparative study of two structurally diverse neurotoxic phospholipases A2, ammodytoxin from the long-nosed viper (Vipera ammodytes ammodytes) and vipoxin from the Bulgarian sand viper (Vipera ammodytes meridionalis) venoms. ДНТС/Словения 01/5, project leader – Prof. S. Petrova
3. Properties, structural and functional characterization of Best1 protein in model membrane systems and epithelial cells, DDVU 02/10, Bulgarian Science Fund, coordinator Assoc. Prof. Dr. J. Doumanov

- BIOBANKING OF SOMATIC AND PLURIPOTENT CELLS

Activities and scientific expertise:

Examination of the reprogramming effectiveness of cell populations from various sources:

- Comparative studies of pluripotent cells from different sources

  Mesenchymal pluripotent cells (bone marrow, umbilical blood, peripheral blood, adipose tissue)

  Embryonic stem cells (intracellular mass)

  Extraembryonic pluripotent (placenta, chorion, umbilical cord)

  Reproductive cells (epithelial, granulosa, germinative)

  Patient-specific somatic cells

- Isolation and characterization of new reprogrammed cell lines

  Examining reprogramming conditions

  Reprogramming methods (chemical, physical, genetic)

  Nutritional environments

  Cryopreservation

  Biomimetics and organoids

- Exploration of functional characteristics of reprogrammed cell lines

  Self-renewal, survival and apoptosis

  Manipulation and control of the cell cycle

  Genomic stability and epigenetics

  Directed differentiation and pluripotent potential

  Phenotypic characterization of biological markers

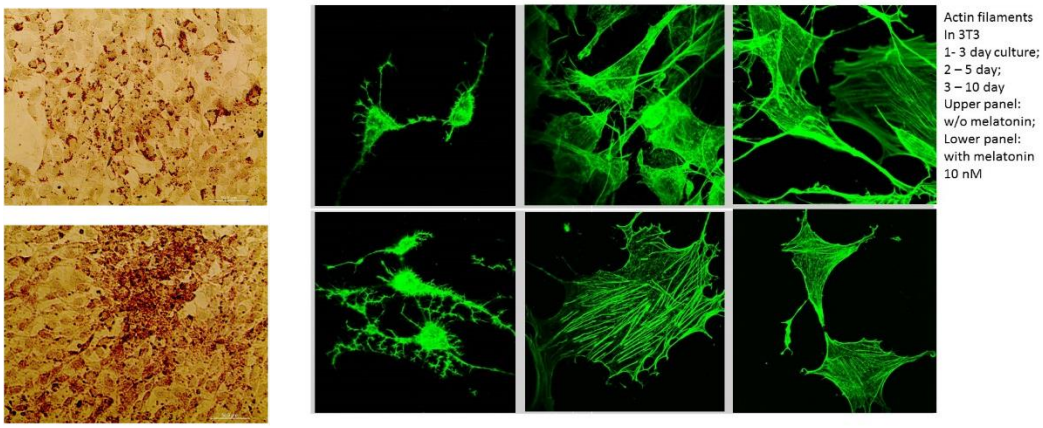
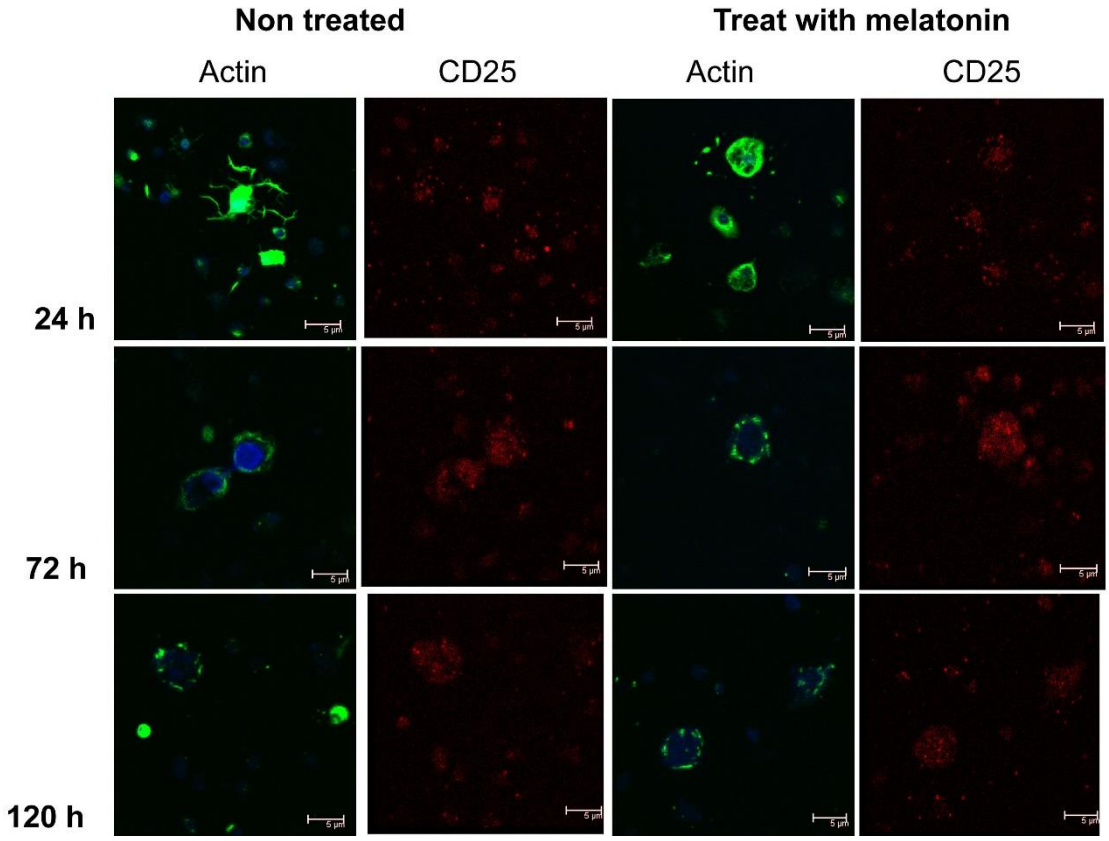


Fig. 8 Adipogenic differentiation of 3T3 mouse embryonic fibroblasts (*Konakchieva et al, 2019)*



Фиг. 9. Effect of in-vitro melatonin on cytoskeletal actin and CD25 + expression. Actin microfilaments (green) and CD25 + (red) expression in non-treated and treated with melatonin cultures of PBMC 24, 72 and 120h post-activation with PHA (5μg / ml) (*Georgiev G. et al. 2018*)

Technical capacity

Available cell lines in the cell bank of the Department of Cytology, Histology and Embryology, Faculty of Biology

Cell line	Origin	Type	Category	Source	Notes
GD25	Mouse	Fibroblast-like	Transformed cell line	Obtained by differentiation from the embryonic stem cell line G201	Integrin β1 knock-out
GD25β1	Mouse	Fibroblast-like	Transformed cell line	Stably transformed clone of GD25	Expressing Integrin β1
	Mouse	Fibroblast-like	Transformed cell line	Transfected with β-actin-GFP GD25 cells	Expressing β-*actin–GFP* protein

GD25 GFP- and RFP-beta actin					
NS0	Mouse	Lymphoblast-like	Tumor	Mouse myeloma	Suspension culture of antibody-producing cells
MDCK / MDCKII	Canine	Epithelial-lie		Canine kidney cells	Polarized epithelial cells
A549	Human	Epithelial-lie	Tumor	Human lung carcinoma	Adherent
hFSC	Human	Epithelial-lie	Tumor	Human fibrosarcoma cells	Adherent
HFF	Human	Fibroblast-like		Human foreskin fibroblasts	Adherent
DPSC	Human	Mesenchymal stem cells		From human molars	
NIH/3T3	Mouse	Fibroblast-like		Mouse embryonic fibroblasts	Adherent
SIRC	Rabbit	Epithelial-like		Rabbit corneal cells	Adherent
HepG2	Human	Epithelial-like	Tumor	Liver carcinoma	Adherent
YAC-1	Mouse	Lymphoblast-like	Transformed cell line	Lymphoma cells	Suspension
SK-Mel-1	Human	Epithelial-like	Tumor	Metastatic cells	Suspension
K562	Human	Lymphoblast-like	Tumor	Leukemia patient-derived cells	Suspension
STO	Mouse	Fibroblast-like		Mouse embryonic fibroblasts - immortalized	Adherent
Jurkat	Human	Lymphoblast-like	Tumor	Leukemia patient-derived T-lymphocytes	Suspension
Skw3	Human	Lymphoblast-like	Tumor	Leukemia patient-derived T-lymphocytes	Suspension
MK2	Monkey (Macaca mulatta)	Epithelial-like		Kidney cells	Adherent
HT1080	Human	Epithelial-like	Tumor	Fibrosarcoma cells	Adherent
RCE	Rabbit	Epithelial-like	Transformed cell line	Rabbit corneal epithelium cells	Adherent
HeLa	Human	Epithelial-like	Tumor	Cervical cancer cells	Adherent

* The cell lines are suitable only for in vitro studies, and not for diagnostic, therapeutic or clinical application

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***Partners**: FCF-Sofia University "St. Kl. Ohridski ", IBIR-BAS, MC Reprobiomed, AG In-Vitro Dimitrov, Biopharma Complex (SofiaTehPark)*

SOFIA UNIVERSITY – FACULTY OF CHEMISTRY AND PHARMACY

Materials with Pharmaceutical and Medical Applications

Biomedical applications of materials are generally of great variety and this is well reflected in the biomedical materials related research performed in the Faculty of Chemistry and Pharmacy, University of Sofia. The related scientific activities are ranging from synthesis of new bioactive substances with potential drug activity, through the synthesis of new dyes for DNA labelling, computational chemistry contribution towards enlightening the structure-activity relationship for compounds with potential biological activity, new ligand complexes influencing the drug activity and drug action in real live systems to finish with polymeric biomaterials used as drug delivery vehicles or as implants combining both very good biocompatibility and mechanical performance. The scientific activities could be divided into following topics:

- Synthesis of new bioactive substances with potential drug activity

- Biologically active coumarin derivatives. New coumarin phosphorus-containing derivatives with high activity as e.g. growth regulators. Here, new synthetic routes for preparation of new bi- and polyfunctional compounds based on coumarins are sought. The research is also focused on enlightening the effect that substituting groups have on the biological activity of the studied organic compounds. The biological activity of the newly synthesized substances is evaluated (Group of Synthesis and Chemical Transformations of Coumarins, Assoc. Prof. R. Nikolova)

- New compounds with antitumor activity. Development of new synthetic methods for synthesis of new classes of heterocyclic derivatives, combining different pharmacophore fragments and investigation of their biological activity. The focus is on stilbene and chalcone derivatives as potential anticancer agents. The bioassay so far show that these compounds exerted cytotoxic effects on various cancer cell lines in a concentration-dependent manner, causing 50% inhibition of the malignant cell proliferation at low micromolar concentrations (Laboratory of Biologically Active Compounds, Assoc. Prof. O. Petrov)

- Complexes based on transition metals with anti-tumor effects. New complexes of biologically active compounds with various metal ions aiming to improve the biological activity of the investigated ligands. For example, coordination properties of some antibiotics applied in veterinary medicine are studied towards biometal ions as Ca, Mg, Cu, Co, Zn, as well as with toxic metal ions such as Cd, Hg. The complex formation by using heavy metal ions is also studied to be applied when acute intoxication occurs in e.g. stock farming. Structure-activity relationship for the new complexes is sought via combined theoretical (quantum-chemical) and spectroscopic studies. Another class of substances is a series of hydantoins and their thio-analogues. Their complexation ability with metal ions, such as Cu(II), Ni(II), Pt(II), Ru(III) is studied with respect to their potential application as anticancer drugs (Laboratory of Biocoordination and Bioanalytical Chemistry (LBBC), Assoc. Prof. I. Pantcheva, Assoc. prof. A. Ahmedova).

- Toxicology. Biochemical and toxicological properties of the vipoxin and its components are under investigation. Analytical toxicology – study on the stability of benzodiazepines in biological samples and their post-mortem biotransformations. The LBBC is the only Laboratory in all Bulgarian universities authorized to perform studies by using narcotics and drugs of abuse. In the Laboratory the following studies in the field of bioanalytical chemistry, toxinology and analytical toxicology can be performed: protein isolation, purification and analysis; analysis of blood (clinical chemistry); testing of newly synthesized compounds and their metal complexes for target inhibition of pathologically significant enzyme systems; snake venom toxins (Vipera ammodytes); toxins affecting blood coagulation and hemotoxicity; analysis of drugs and metabolites in biological samples; stability of drugs in biological samples and post-mortem biotransformations (Laboratory of Biocoordination and Bioanalytical Chemistry (LBBC), Assoc. Prof. V. Atanasov, assist. prof. S. Stoykova).

- Biologically active heterocyclic compounds. Synthesis of mono- and bicyclic lactams. Introduction of a peptide bond or another heterocycle in the side chain to the tetrahydroisoquinolinone, piperidinone and pyrrolidinone ring. Synthesis of isochromanones and dibenzochromenones. (Lab on Chemistry of heterocyclic compounds, Assist. Prof. Dr. Nikola Burdzhiev).

- Biologically active substances with antibacterial and antifungal activity. Series of 3,4-disubstituted 3,4-dihydroisocoumarins have been synthesized and their antimicrobial acticity was studied. Synthesis of new heterocyclic compounds incorporating aminoacids for preparation of new ACE inhibitors. Their antibacterial and antifungal activity is investigated in collaboration with the Faculty of Biology, University of Sofia (Lab on Chemistry of heterocyclic compounds, Prof. Dr. Milen Bogdanov)

- Biologically active substances from nature. Materials extracted from natural sources with different biological effects. "Green" methods for extraction of biologically active substances. (Lab on Chemistry of heterocyclic compounds, Prof. Dr. Milen Bogdanov)

➢ Materials for biomedical applications

- New dyes for DNA binding. Development of novel fluorescent nucleic acid probes. Studies on the organic dyes binding with DNA in order to design novel and more efficient drugs targeted at DNA and to explore the biological function of nucleic acids and the interaction mechanisms with some drugs. Intercalating fluorescent non-covalently binding nucleic acid probes. Development of novel, more environmentally friendly, methods to prepare monomethine cyanine dyes and to improve known synthetic procedures used to obtain intermediates. New dyes suitable for PCR (minor groove binders). New symmetrical and asymmetrical monomethine cyanine dyes which absorb in the ultraviolet spectrum and can be excited by cheap light sources at 405 or 445 nm (Lab for Dye Synthesis, Assoc. Prof. A. Vassilev)

- Mechanics and dynamics of the bilayers, membranes and cells adhesion. Study of the state, electrical and rheological properties of biological model systems as monolayers, liposomal bilayers and suspensions. Detailed physicochemical study of important for the pharmacy, medicine and biotechnology model systems of lipid and protein nanostructures. Specific systems for study: the interfacial and temporal organization of the lipolysis; mechanisms of degradation of the polyesters, lipids and proteins; mechanisms of formation and degradation of micro- and nanocapsules with potential applications in pharmacy; photochemical reactions at the interface; state, rheological and electrical properties of the biopolymer model monolayers from the plant kingdom; thin liquid films. Atomic Force Microscopy (AFM) characterization of molecularly ordered nanostructures and its applications in (bio)catalysis. (Laboratory of Biophysical Chemistry, Prof. Tz. Ivanova, Prof. K. Balashev).

- Biocompatible polymers as implants and drug delivery systems. Exploitation of the unique biocompatibility of zwitterionic polymers (PZ). Development of biomaterials (hydrogels, copolymers, double networks, etc.) based on PZ. Correlation between the antipolyelectrolyte effect and PZ non biofouling properties is sought. PZ based materials with applications in cosmetics, as soft contact lens,

biolubricants, coronary vascular prosthesis, catheters, haemodialyses membranes, angiosurgery stents, etc. The application of the polyzwitterions as materials against amyloid-fibril formation at neurodegenerative diseases. Novel PZ copolymer latexes with nano-size synthesized by an emulsifier-free emulsion copolymerization and their application for sustained drug release (Metoprolol tartrate and Verapamil hydrochloride) (Laboratory on Water-Soluble Polymers, Polyelectrolytes and Biopolymers, Assoc. Prof. E. Kamenska, Assoc. Prof. L. Hristov)

- Polymeric implants and scaffolds for regenerative medicine. Biocompatible polymer materials and hydrogels. Polymer hydrogels for wound healing management. Interpenetrating polymer networks (IPN) as implants and scaffolds for tissue engineering. Hybrid polymer/calcium phosphate materials for dentistry. Structure-properties relationship for polymeric materials revealed by HRMAS NMR (Laboratory on Structure and Properties of Polymers, Assoc. Prof. E. Vassileva)

- Polymeric drug delivery systems and scaffolds/implants. Polymer and proteinaceous nano- and microcapsules/particles as delivery system for therapeutic agents (hydrophobic drugs, etc.). Structure-properties relationship for polymeric materials (Laboratory on Structure and Properties of Polymers, Assoc. Prof. E. Vassileva, Assist. Prof. Marin Simeonov)

- Enabling formulations for delivery of poorly water-soluble drugs and in vitro digestion studies. The solubility enhancement of hydrophobic drugs by using surfactants, saponins, phospholipids and polymers is studied in the context of parenteral and oral drug delivery. The mechanisms of the solubilization process and the structure-activity relationship of the drug-excipient combinations are particularly investigated by using dynamic light scattering (DLS) and ^{1}H NMR diffusion-order spectroscopy (DOSY). The interactions of pharmaceutical excipients (e.g. surfactants) with the bile salts present in biorelevant dissolution media, and their impact on drug solubility and precipitation in conditions close to the real in vivo situation are also studied. An in vitro digestion model that has been developed in-house and has been validated with in vivo studies is also available. The in vitro digestion model allows the assessment of (1) enzyme activity, (2) the physicochemical changes in food and drug formulations and (3) the solubility and stability of bioactive molecules in conditions mimicking the gut. (Laboratory for active formulations and materials, Prof. N. Denkov, Prof. S. Tcholakova, Assist. prof. Z. Vinarov)

- Excited state relaxation dynamics of organic and inorganic materials for optoelectronic devices. Those include compounds for OLEDs, optical chemosensors and photovoltaics. Investigations are related to excited state energy redistribution and relaxation dynamics of various metal organic complexes with electron transporting properties employed as emitting layer in organic light emitting devices. Examples are Aluminum, Europium and Iridium complexes with various organic ligands as 1,3-diketonates, Phenyl Benzothiazoles and Schiff bases. Studies aiming determination of thermodynamic properties of photoinduced net-reactions in intelligent compounds, such as substituted flavylium salts. Study of charge generation, injection and recombination dynamics of materials for photovoltaic applications, as hybrid organic-inorganic Led Perovskites and quantum dots. (Steady state and time resolved electron spectroscopy lab, Assist. Prof. Dr. Stanislav Stanimirov).