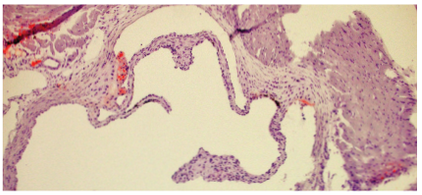
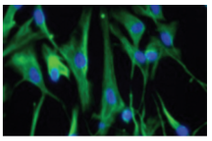
DEPARTMENT OF BIOPATHOLOGY AND THERAPY OF INFLAMMATION





***Ileana Mânduțeanu**, PhD*
HEAD OF DEPARTMENT

INFLAMMATION RESEARCH LABORATORY

STAFF

***Elena Butoi**, PhD*
HEAD OF INFLAMMATION RESEARCH LABORATORY
***Monica Țucureanu,** PhD*
***Ana Maria Gan,** PhD*
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***Răzvan Macarie,** PhD student*
***Letiția Ciortan,** PhD student*
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***Gabriela Meșca,** Technical assistant*

MEDICAL AND PHARMACEUTICAL BIONANO-TECHNOLOGIES LABORATORY

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***Elena Valeria Fuior,** PhD student*
***Geanina Voicu,** PhD student*
***Maria Anghelache,** Master student*
***Marilena Misici,** Technical assistant*

FORMER RESEARCH STAFF:
Maria Calb,Cristina Lupu, Florea Lupu, Aurelian Radu, Geo Șerban, Viorel Simion.

CORE LABORATORY UNITS:
Cell Adhesion
responsible: Elena Butoi
Exploratory Imagistics
responsible: Florentina Safciuc

DEPARTMENT OF BIOPATHOLOGY AND THERAPY OF INFLAMMATION

Ileana Mânduțeanu, *PhD*

Head of Department
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Major position/appointments

- Deputy Director
- Associate member of the Romanian Academy
- Member of The Romanian Academy of Medical Sciences
- Member of the Scientific Council of ICBP "N. Simionescu"
- PhD Advisor in Biology
- Expert evaluator of the national and international grants
- Peer reviewer at national and international journals

MAJOR RESEARCH INTERESTS

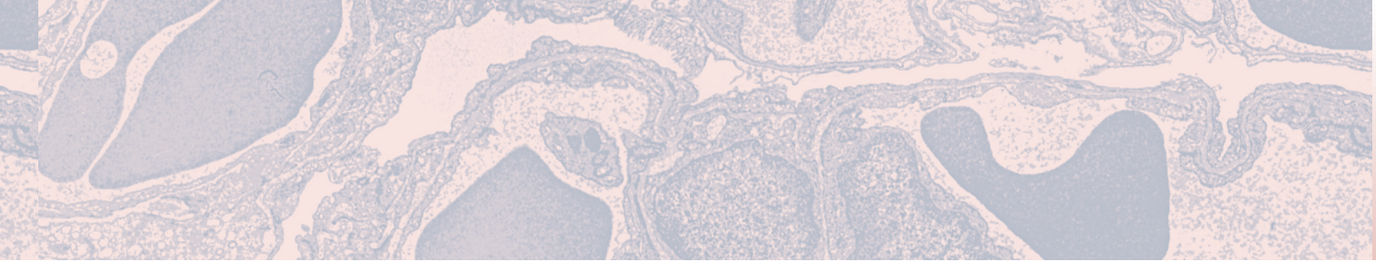
- **To identify the specific mechanisms of valvular disease progression and the development of new nanobiotherapeutics for diabetes-aortic valve disease**
- **To identify relevant and specific biomarkers for vascular inflammation associated with atherosclerosis and diabetes as targets for nanotherapy**
- **Design of novel drug delivery systems to specifically target inflammation**

PUBLICATIONS

Over 80 original articles (>1100 citations) were published in Web of Sciences Core Collection journals and 5 book chapters between 1979-2019 by researchers of the Department.

PREVIOUS PROJECTS RELEVANT PUBLICATIONS

- **Surface alteration of blood platelets in diabetes mellitus** *(Lupu C, Calb M, Atherosclerosis, 1988; C Lupu et al., Platelets, 1992; J Mol Cell Cardiol, 1993; Platelets, 1994).*
- **Structure and function of valvular endothelial cells in normal and pathological conditions** (*Mânduțeanu I. et al., J Mol Cell Cardiol, 1988).*
- **Interaction of valvular endothelial cells with blood cells** *(Mânduțeanu I. et al., J SubmicroscCytolPathol, 1992; Lupu C. et al., Platelets, 1993; Mânduțeanu I. et al., Endothelium, 1999).*
- **The use of liposomes as drug delivery carriers** *(Voinea M. et al., Vascular Pharmacol, 2002; Voinea M. et al., J Cell Mol Med, 2002; Voinea M. et al., Eur J Pharmacol, 2004; Voinea M. et al., Pharm Res, 2005; Voinea M, Călin M. et al., Cell Tiss Res, 2009; Țucureanu MM et al., Int J Nanomedicine, 2017).*
- **Mechanisms involved in the effects of anti-inflammatory drugs on activated endothelial cells** *(Mânduțeanu I. et al., Pharmacology, 2002; Eur J Pharmacol 2003, Mânduțeanu I et al. Pharmacology, 2007; Dragomir E. et al., J Diab Complications, 2004).*
- **Modulation of MCP-1 and fractalkine expression by high glucose conditions in vascular cells: effects of anti-inflammatory drugs** *(Dragomir E. et al., Vascular Pharmacol, 2006; Dragomir E. et al., Thromb Haemost, 2008).*



- **Molecular links between chronic inflammation and accelerated atherosclerosis: role of resistin and chemokines (fractalkine and CXCL16); new avenues for targeted therapy** (*Mânduțeanu I et al,Biochemical and Biophysical Research Communications, 2009*; *Manduteanu I et al, Biochemical and Biophysical Research Communications, 2010*; *Stan D et al, Cell Tissue Res, 2011).*
- **Molecules and mechanisms involved in cytokine and chemokine-dependent vascular inflammation as targets for novel nanotherapeutic strategies** *(Butoi ED et al, 2011,Biochim Biophys Acta;Manduteanu I, Simionescu M,2012, J Cell Mol Med; Pîrvulescu M et al, 2012, Biochemical and Biophysical Research Communications Journal;Gan AM et al,2013, Cell Tissue Res*; *Gan AM et al, 2013, J Cell Biochem;Pirvulescu MM et al, 2014, Int J Biochem Cell Biol*.; *Gan AM et al, 2014, FEBS J.; Butoi E et al, 2014, Crit Rev Eukaryot Gene Expr; Simion V et al., Mediators Inflamm, 2016; Butoi E et al.,BiochimBiophys Acta, 2016, Țucureanu MM et al., Cytokine, 2016).*
- **Nanoparticles designed to target chemokine-related inflammatory processes in vascular diseases and cancer metastasis** (*Simion V et al., Journal of Nanoparticle Research, 2013; Calin M et al., Eur J Pharm Biopharm, 2015, Roblek M et al., J Control Release, 2015, Schlesinger M et al., Int J Clin PharmacolTher, 2015, Calin M, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2012, Călin M et al., European Patent no. EP 2832373*).
- **Toxicological studies and the inflammatory response induced by the exposure of human cells to Ag/TiO_2 nanoparticles developed for leather surface functionalization** (in collaboration with National Institute for Research and Development of Textiles and Leather, Bucharest Romania and Minho University, Portugal) (*Rebleanu D et al., Toxicology, 2019; Rodino S et al.,Banat's Journal of Biotechnology, 2017, European Patent: application no: 17464014.4-1102, OSIM patent application no. A/00966).*
- **Investigation of cytotoxicity and gene transfection ability of non-viral vectors obtained by covalent coupling of hyperbranched PEI chains (Mw 2 kDa) with different core molecules using cultured cells** (in collaboration with Institute of Macromolecular Chemistry "Petru Pomi, Iasi, Romania) (*Uritu CM et al., J. Mater. Chem. B, 2015a; Uritu CM et al., J. Mater. Chem. B, 2015b; Marin L et al., ACS Biomater. Sci. Eng., 2016; Dascălu AI et al., J. Mater. Chem. B, 2017; Simionescu BC et al., Mater. Sci. Eng. C, 2017; David G et al, Polym. Chem., 2018*).
- **MicroRNA signature of vascular cells cross-talk relevant for the atherosclerotic plaque rupture in patients with type II diabetes** (*Macarie RD et al., 2018*)

PATENTS

- European Patent no. EP2832373, inventors Bendas G, Borsig L, Calin M, Cevher E, Enachescu M, Gok MK, Hoffmann A, Mihaly M, Pabuccuoglu SK, Simionescu M, Schlesinger M, Zeisig R: "*Liposome for blocking site-specifically chemokine-related inflammatory processes in vascular diseases and metastasis*"
- European Patent: application no: 17464014.4-1102, inventors: Gaidau C, Călin M**,** Constantinescu CA, Rebleanu D, Stoica T: "*Leather with anti-microbial and self-cleaning properties and process for obtaining therof*"
- OSIM, application no. A/00966, inventors: Gaidau C, Călin M, Constantinescu CA, Rebleanu D, Stoica: "*Leather with anti-microbial and self-cleaning properties and process for obtaining therof*"
- OSIM application no A/00811, inventors Călin M, Rebleanu D, Constantinescu CA, Voicu G, Deleanu M, Mânduțeanu I: "*Process for obtaining the nanocarriers for targeted delivery of interference ribonucleic acid (RNA) to aortic valve cells*"
- OSIM application no A/01055, inventors: Ficai D, Ardelelean I, Ilie C, Călin M, Fuior EV, Fifere A, Pinteală M, Fundueanu-Constantin G, Ficai A, Simionescu M, Andronescu E: "*Vertical magnetic (electro) separator of isomagnetic nanoparticles*"

Elena Butoi, *Ph.D.*

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INFLAMMATION RESEARCH LABORATORY

Major position/appointments

- Principal Investigator, Scientific Researcher grade I
- Supervision of Graduate Students and Postdoctoral Fellows
- Expert evaluator of the national grants
- Invited Peer Reviewer for International Scientific Journals

STAFF

Monica Țucureanu, *PhD* / ***Ana Maria Gan,*** *PhD* /
Sergiu Cecoltan, *PhD student* / ***Mihaela Vădana,*** *PhD student* /
Răzvan Macarie, *PhD student* / ***Letiția Ciortan,*** *PhD student* /
Andreea Mihăilă, *Master student* / ***Gabriela Meșca,*** *Technical assistant*

SELECTED NEW FINDINGS OF THE LABORATORY

MOLECULAR AND CELLULAR MECHANISMS OF CARDIOVASCULAR DISORDERS

• Enoxaparin reduces monocyte adhesion to TNF-α, LPS-, or high glucose-activated EC

• Aspirin corrects the high glucose-induced changes in intracellular calcium homeostasis and NO production in human EC

• PPARα activators (fenofibrate and clofibrate) inhibit MCP-1 and fractalkine expression induced by high glucose in human smooth muscle cells

• Identification of early structural and functional changes induced by diabetes in the aortic valve in vivo and establishing significant correlations between inflammatory, remodeling and calcification markers and functional and serum parameters

• Development of a 3D construct of human aortic valve as a model to study calcification mechanisms in aortic valve disease

• In diabetic conditions, upon cross-talk with macrophages, smooth muscle cells exhibit augmented expression of MMP-1 and MMP-9 and higher levels of soluble MCP-1 which is functionally active and involved in MMPs regulation

• Cross-talk between macrophages and smooth muscle cells amplifies inflammation in macrophages, impairs collagen and metalloprotease synthesis and promote angiogenesis

• Functional analysis of the fractalkine gene promoter in human aortic smooth muscle cells exposed to proinflammatory conditions

• Subendothelial resistin enhances monocyte transmigration in a co-culture of human endothelial and smooth muscle cells by mechanisms involving fractalkine, MCP-1 and activation of TLR4 and Gi/o proteins signaling

• Monocytes and smooth muscle cells cross-talk activates STAT3 and induces resistin and reactive oxygen species production.

• Resistin has pro-inflammatory effects on human smooth muscle cells: up-regulates fractalkine and its receptor, CX3CR1 expression by TLR4 and Gi-protein pathways.

• A novel pro-inflammatory mechanism of action of resistin in human endothelial cells: up-regulation of SOCS3 expression through STAT3 activation.

INFLAMMATION RESEARCH LABORATORY

CURRENT PROJECTS

1. TARGETED THERAPIES FOR DIABETES - RELATED AORTIC VALVE DISEASE – THERAVALDIS, A MULTI-LABORATORIES PROJECT

Aortic valve disease and especially calcific aortic valve disease (CAVD) is a global health burden in all aging societies, including the Romanian population. It is known that the presence of diabetes accelerates CAVD, and is predictive of poor prognosis in valve disease and of faster degeneration of implanted bio-prosthetic aortic valves. To our knowledge, a clinically viable pharmacological therapy for valve disease is still not available, the only alternative being the invasive and costly valve replacement. This urges the need for additional research to identify distinctive mechanisms of valve disease progression.

THERAVALDIS' objective is to advance the understanding of the mechanisms of aortic valve disease in the diabetic milieu in order to discover and validate new possible targets for nanotherapy and stem cell therapy;

Hypothesis: that upon the identification of distinct diabetes-related changes in valvular cells and matrix components from relevant in vivo models, we could target the major alterations with appropriate therapy as treatment for valve diseases. The three **main pathogenic processes** envisioned as putative targets are: endothelial to mesenchymal transition (EndMT), ectopic osteogenesis in the valves and dysfunctional recruitment and homing of progenitor cells.

Ongoing experiments are focused on:

• Characterization of the early and progressive changes induced by diabetes in the aortic valves in vivo, as well as in the circulating endothelial progenitor cells (EPC). This will advance the understanding of aortic valve disease and will indicate new possible biomarkers and new targets for therapies.

• Developing of in vitro 3D models of aortic valve leaflet seeded internally with human valvular interstitial cells (VICs) and externally with human valvular endothelial cells (VECs) to validate the specific terapeutic targets revealed by our results.

• Tests and validation of new therapeutic strategies: (a) *targeted nanotherapeutics*;(b) *stem cell therapy*

• Preclinical validation of the most efficient nano and/or stem cell-based therapies *in vivo*.

Methods employed and Results obtained:

We evaluated changes of aortic valve function and changes associated with inflammation, ECM-remodelling, and calcification induced in a hyperlipemic ApoE-/- mouse model by early type I diabetes onset (at 4 and 7 days after streptozotocin induction).

The hemodynamic valve parameters were evaluated by echography and blood samples and aortic valves were collected.

Plasma parameters were measured and inflammatory, remodelling and osteogenic markers were evaluated in the aortic valves. Correlations between all parameters were determined.

MAIN FINDINGS :

• **Diabetes induces early functional alterations in the aortic valves of hyperlipemic ApoE-/- mice.** The results revealed that the mean value of velocity time integrals (VTI) was significantly increased both for D4 group compared to C4 group (by ~1.56 times,) and for D7 group compared to C7 group (by ~1.77 times) and the mean value of transvalvular velocity (VEL) was significantly higher for D7 group vs C7 group (by ~ 1.80 times).

• **The aortic valves of hyperlipemic ApoE-/- diabetic mice exhibit increased expression of inflammatory markers:P-selectin, ICAM-1, VCAM-1, PECAM-1 expression, as well as TGFβ family members: TGFβ1, BMP2, and BMP4.**

• The aortic valve of hyperlipemic ApoE-/- diabetic mice display enhanced expression of pro-osteogenic markers: alkaline phosphatase (ALPL), osteopontin and osteocalcin, cell activation markers, fibronectin and remodeling molecules: MMP2 and MMP9

• Correlations between aortic valve tissue markers, plasma parameters and hemodynamic parameters in hyperlipemic ApoE-/- diabetic mice were established

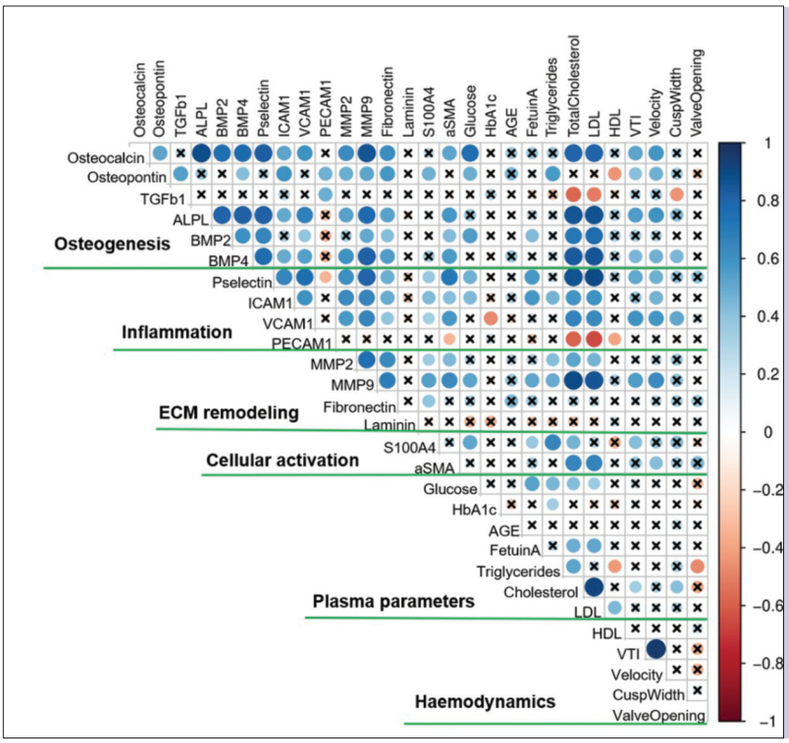
To gain insight into the possible associations between hemodynamic parameters, tissue markers and plasma parameters determined in this experimental setting, we performed a correlation analysis.Our analysis revealed that:

• peak aortic jet velocity was highly correlated with inflammatory biomarker VCAM-1, pro-osteogenic markers osteocalcin and ALPL, remodeling enzyme MMP9 and myofibroblast marker αSMA;

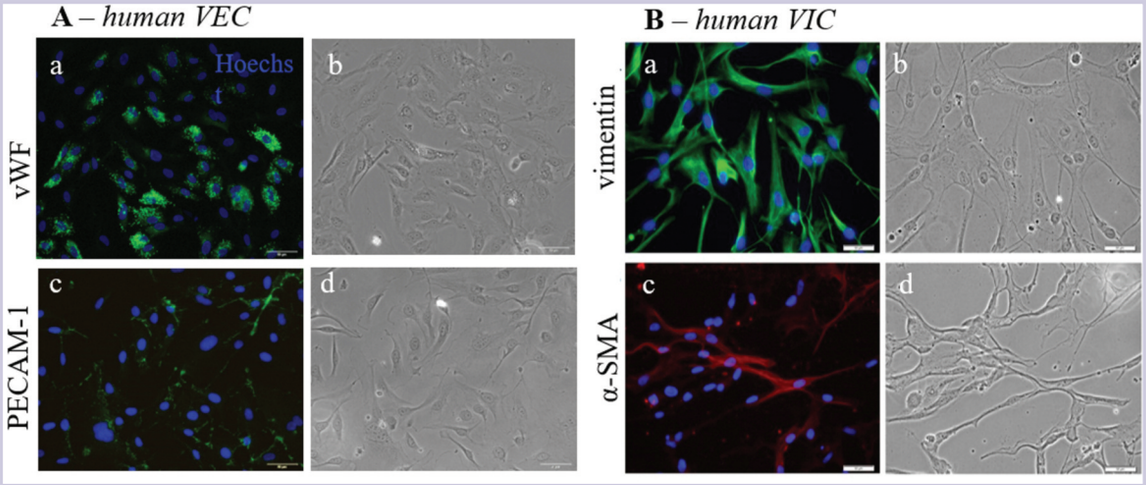
• glycemia was significantly correlated with: osteocalcin, osteopontin, BMP2, P-selectin, ICAM-1, MMP9, S100A4, Fetuin A, triglycerides, total cholesterol and LDL-cholesterol.

• very high correlations ($r > 0.8$) were found between: P-selectin and plasma parameters: LDL and total cholesterol, osteoblastic markers osteocalcin and ALPL and remodeling enzyme MMP9; BMP2 and ALPL, BMP4 and total cholesterol, LDL, ALPL and MMP9; MMP9 with total cholesterol, LDL, P-selectin; ALPL with total cholesterol, LDL, P-selectin, BMP4, osteocalcin;

• high correlations ($0.5<r<0.8$) were found between: P-selectin with Fetuin A, VCAM-1, ICAM-1, BMP2, BMP4, MMP2, α-SMA, VTI; VCAM-1 with total cholesterol, LDL, P-selectin, ICAM-1, BMP4, osteocalcin, ALPL, MMP9, MMP2, αSMA and the hemodynamic parameters VTI and velocity. ICAM-1 displayed a positive high correlation with total cholesterol, LDL, Fetuin A, P-selectin, VCAM-1, osteopontin, osteocalcin, with ALPL, MMP9, MMP-2 and with fibronectin. PECAM-1 displayed a negative high correlation with the seric parameters total cholesterol and LDL.



Correlation matrix between pro-osteogenic, inflammatory, ECM remodelling, cellular activation, plasmatic and hemodynamic parameters. *Mean values of measured parameters for each experimental group were used for a Pearson correlation matrix conducted using the R package "corrplot". Blue represents positive correlation; red represents negative correlation; darker colours.*

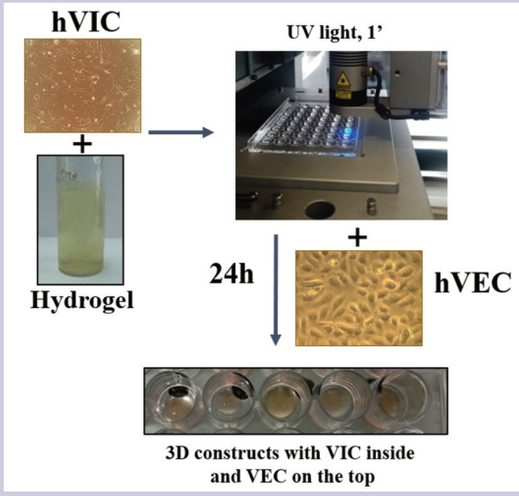


Expression of valve endothelial (A) and interstitial (B) cells specific markers in 2D culture; obtaining of the 3D-construct. ***A.*** *Positive fluorescent staining for (a) von Willebrand Factor and (c) CD31(PECAM-1) in the human aortic valve endothelial cells isolated from human operated valve. b, d– contrast phase images.* ***B.*** *Positive fluorescent staining for (a) vimentin and (c) alpha-smooth muscle actin (α-SMA) of human aortic valve interstitial cell. DAPI was used as a counterstain; scale bar=50 μm.*

Development of an *in vitro* 3D model of human aortic valve leaflet.

To develop 3D in vitro models**,** initially, we isolated and characterized the human valvular endothelial cells (VECs) and valvular interstitial cells (VICs) from human aortic operated valves. Aortic VECs are positive for endothelial markers von Willebrand factor (vWF) and PECAM-1 and VICs are positive for α-SMA and vimentin protein.

After cells isolation and characterization, human VEC and VIC were used for development of a 3D construct similar with human valve leaflet for study of calcific valve disease. Thus, VICs were encapsulated in a methacrylated gelatin-based hydrogel (GP-MA) followed by exposure for 1 minute to UV light for crosslinking. Subsequently VECs were cultivated on topand the 3D constructs were placed in DMEM and cultured for different period of time.

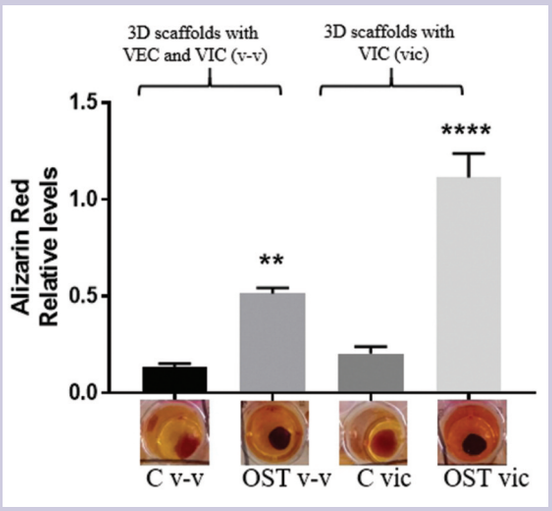


3D-construct

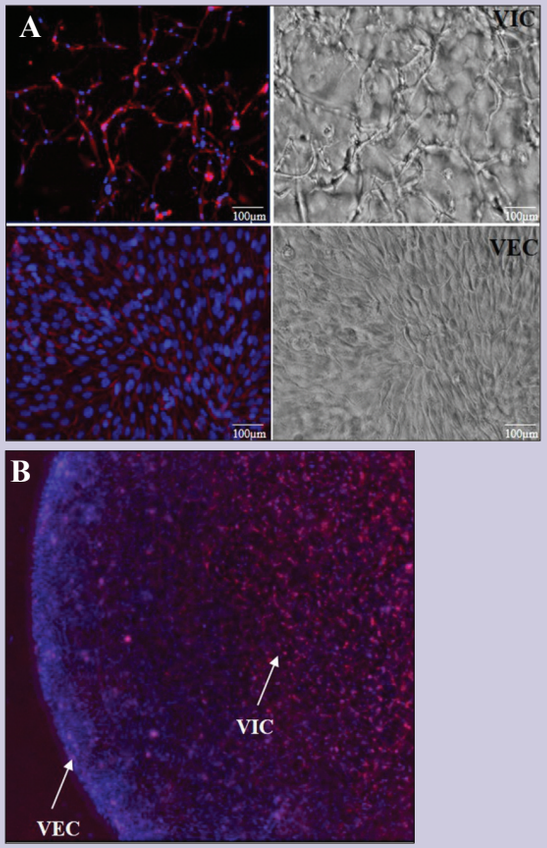
Human VICs were re-suspended in GP-MA hydrogel solution (gelatin methacrylate (10%), 1% alginate and Photo-initiator - Igracure 2595). 100 μL of the VICs-laden prepolymer solution was drop-wise added on a sterilized glass side covered with a 48-hole (Ø - 8 mm, 1 mm thickness) and subsequently crosslinked by exposure for 1 minute to UV light (365nm) using 3DDiscovery® bioprinter (RegenHu). Cell-laden hydrogels were removed from the glass slide and cultured, according to protocol. After 24h, VECs (5x10^5/cm^2) were added on the top of 3D constructs.

Started with the second day, encapsulated VICs in 3D constructs started to gain a fibroblast-like phenotype with elongated-shape appearance, and were homogeneously distributed through the entire hydrogel thickness. VECs cultured on top of the 3D construct grow, proliferate and form a monolayer over the hydrogel, as show in the phase contrast picture or TRITC-phalloidin and Hoechst staining of construct.

Functionality of our 3D model to study the mechanisms of calcific valve diseases was tested by exposure of 3D constructs with human VICs or with VEC-VIC to osteogenic stimuli for 14 days. Alizarin Red experiments revealed the calcific nodule formation (increased calcium staining) in 3D constructs exposed to osteogenic media compared with constructs grown in normal culture media.



Osteogenic environment activates VICs in 3D constructs. A. *Alizarin red was used to stain the mineralized nodules formed by cells from 3D constructs with VIC or 3D constructs with VEC and VIC (v-v) cultured for 14 days. The lower panel showed the representative stained construct from each of the experimental group. The upper graph displayed the quantitative measurement of Alizarin red dye released from the mineralized nodules formed in 3D constructs cultured in normal conditions or exposed to osteogenic media (OST). n = 3, **p < 0.001, ****p < 0.0001.*



Morphology of VICs and VECs from 3D constructs.

A*. The cell morphology in 3D construct at day 7 of culture as determined by phalloidin labelled F-actin (red) and DAPI nuclear staining (blue) – left side. Hydrogel formula supports cell network development of VICs (inside the hydrogel) and VECs proliferation as a monolayer on the scaffold surface. Contrast phase images of the surface and inside of hydrogel with VICs encapsulated inside and VECs cultured on top - right side. Scale bar indicates 100 µm.*

B: *3D reconstruction of multiple images of a 3D construct, realized by z-stack option of cellSense software – median image.*

Ongoing experiments investigate molecular mechanism of valvular calcification in 3D construct exposed to diabetic conditions (high glucose concentration).

2. TARGETING INNATE IMMUNE MECHANISMS TO IMPROVE RISK STRATIFICATION AND TO IDENTIFY FUTURE THERAPEUTIC OPTIONS IN MYOCARDIAL INFARCTION

(INNATE-MI, a multi-laboratories Project; Coordinator: Maya Simionescu, Elena Butoi - responsible for Objective 1 implementation and for project management).

Myocardial infarction (MI) is a major cause of morbidity and mortality. At present, clinicians lack specific biomarkers for accurate post-MI risk stratification and therapeutic tools to modulate myocardial inflammation and to promote efficient recovery. Innate immune processes mediated by polymorphonuclear neutrophils (PMN) and macrophages (MAC) in the immediate post-MI period determine the extent of myocardial damage but also induce repair. Our major goal is to identify central molecules that mediate the crosstalk between sub-populations of PMN and MAC, and determine their involvement in MI. Additionally, we will test the ability of specific therapies to regulate myocardial inflammation and to improve cardiac function in-vivo.

The expected outcome is to identify biomarkers that can be used to accurately identify patients at high risk to suffer new events.

One of the important objectives of the multi-laboratories project is: **To identify immune mediators involved in the dynamic crosstalk between PMN and MAC sub-populations that modulate the inflammation/repair balance post-MI**–where we are investigating the relationships between the soluble mediators produced by PMN/MAC, with post-MI cardiac function and prognosis in MI patients.

THE SPECIFIC ACTIVITIES INCLUDE:

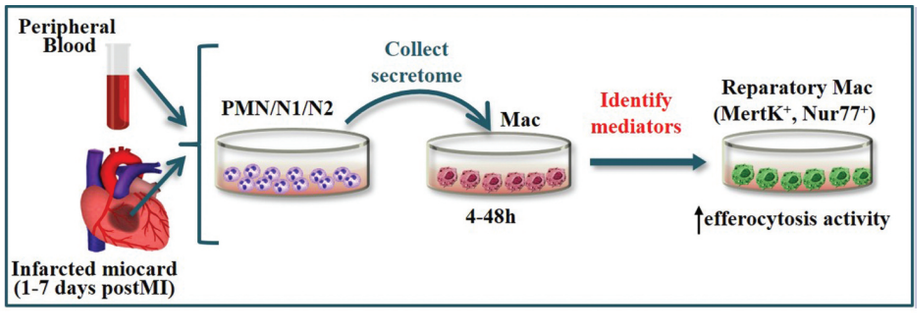
I) isolation of mouse and human polymorphonuclear neutrophils (PMN)

II) polarization of PMN towards N1 and N2 phenotype by exposure of isolated PMN to LPS and IFNγ for N1 and with IL-4 for N2. Type 1 PMN - N1, predominate in the inflammatory phase of an acute ischemic event, and express the pro-inflammatory proteins IL-1β, IL-12 and TNFα. In contrast, type 2 PMN (N2) are present in the reparatory phase (their presence gradually increases in the myocardium during the first week post-MI), and preferentially express the anti-inflammatory mediators IL-10 and TGF-β.

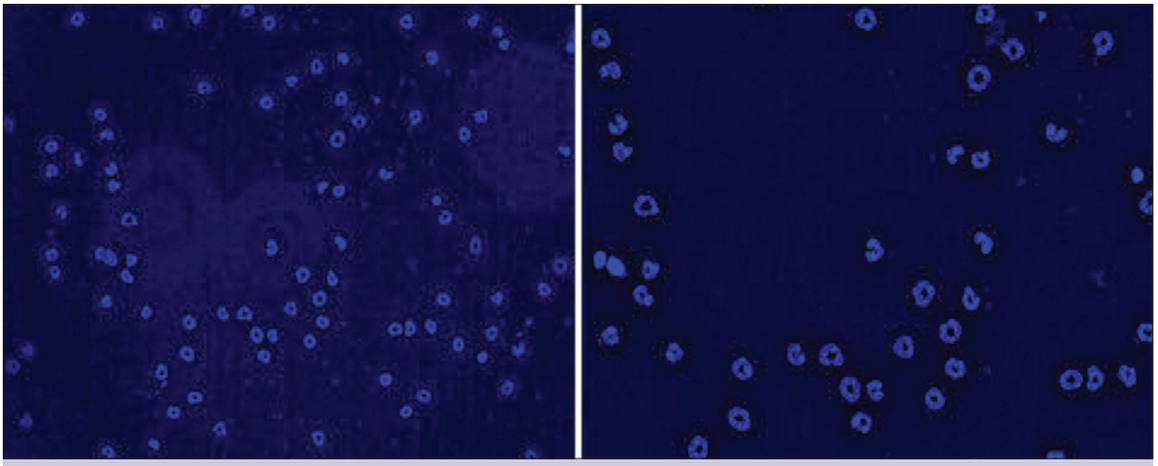
III) obtaining the N1 and N2 secretome that will be further investigated for its effect on macrophage polarization.

RESULTS:

► Isolation of a 98% pure neutrophil population of mouse neutrophils and polarization to obtain N1 and N2 neutrophil phenotypes.



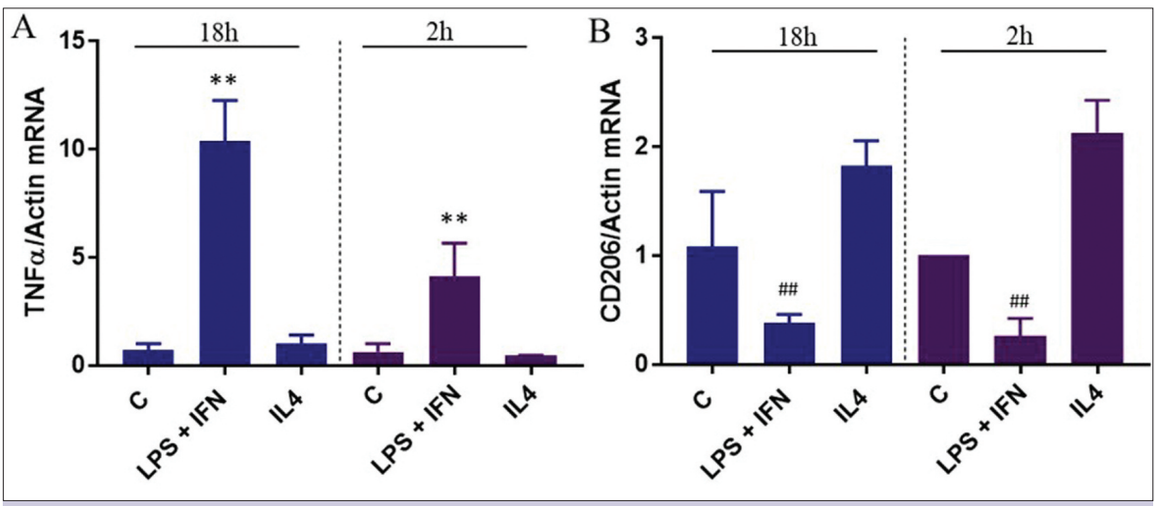
Schematic representation of the experimental procedure of the objective.



DAPI staining of neutrophils isolated from bone marrow.

Therefore, our recent data indicate that exposure of neutrophils for 2h or 18h to 100 ng/ml lipopolysaccharide (LPS) and 20 ng/ml interferon gamma (IFNγ) or 20 ng/ml interleukin 4 (IL-4) led to polarized N1, respectively N2 neutrophil phenotypes, as demonstrated by expression of N1 specific marker - TNF-α when cell were exposed to LPS with IFNγ and N2 specific marker - CD206 when cell were exposed to IL-4.

Ongoing experiments are designed to further investigate the specific inflammatory/ anti-inflammatory markers specific for N1 and N2 and to analyse the impact of neutrophil secretome on macrophage polarisation towards reparatory Mac phenotype.



Activation of neutrophils with LPS+IFNγ induced inflammatory marker TNF-α gene expression (A), while exposure to IL-4 induced anti-inflammatory marker CD206 (B).

3. INTELLIGENT THERAPIES FOR NON-COMMUNICABLE DISEASES BASED ON CONTROLLED RELEASE OF PHARMACOLOGICAL COMPOUNDS FROM ENCAPSULATED ENGINEERED CELLS AND TARGETED BIONANOPARTICLES

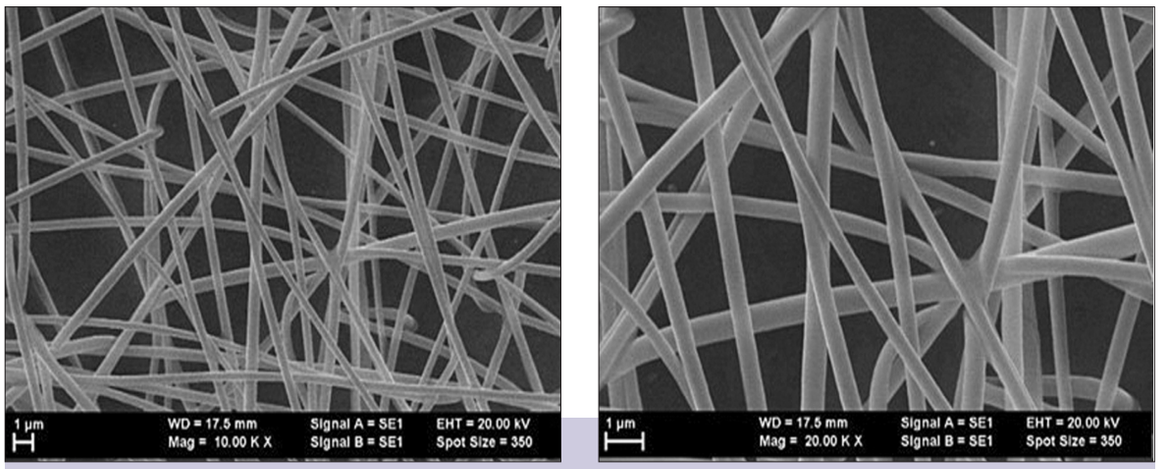
(complex project coordinator:*Maya Simionescu*), INTERA2 component project: "Development of a 3D platform designed for pre-clinical drug testing composed of cells incorporated into three-dimensional bio-matrices", project responsible: *Elena Butoi*)

Non-communicable diseases (atherosclerosis, diabetes, obesity), a major cause of mortality, are characterized by associated inflammatory processes. Valvular diseases represent an important health problem affecting people of all ages, and to date there is no drug therapy for this pathology. The two-dimensional (2D) cultures of valvular cells cannot simulate or mimic the complexity of cardiac valve tissue, derived from interaction between cell types and matrix elements, essential for proliferation, differentiation, morphology, gene and protein expression, and cellular response to external stimuli. As a result, many of the drugs tested in 2D culture conditions fail during clinical trials, especially during Phase III, which is the most expensive clinical phase. 3D culture systems not only provide cell-cell and cell-MEC interactions to study cellular behaviours imitating in vivo conditions, but also offer the opportunity to co-culture multiple cell types to closely mimic tissue in vivo, being suitable for both drug discovery and tissue engineering. Therefore, the complex project INTERA-2 aims to develop a 3D platform designed for pre-clinical drug testing composed of cells incorporated into three-dimensional bio-matrices.

The aim of project 2 is to create a three-dimensional biological (3D) platform by cellular electrospinning/cryogenation with a similar structure to the valvular sheet, for pre-clinical testing of drugs for heart valve diseases.

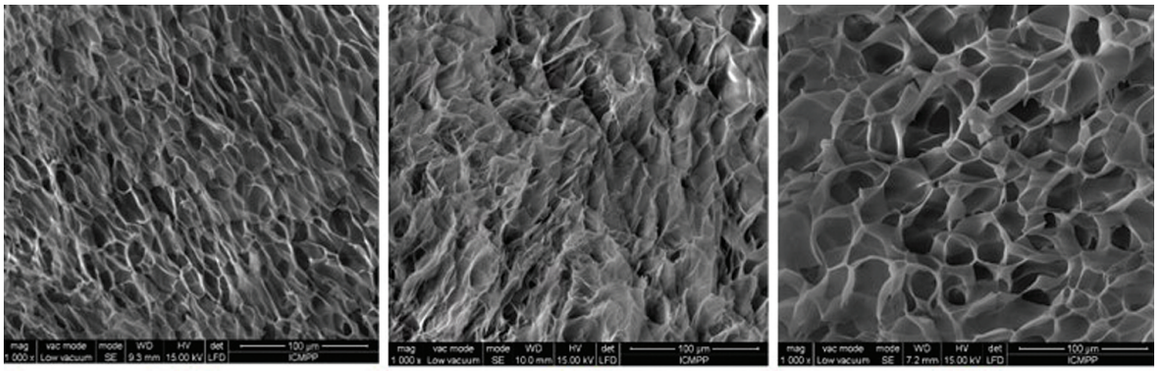
RESULTS:

► **Development of 2 different scaffolds: electrospinning scaffold and cryo-genated hydrogel and cultured human valvular cells on their surfaces.**



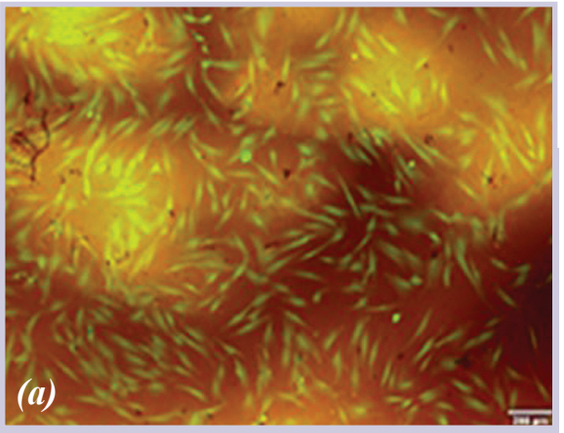
SEM images of electrospun PMMA fibers in dimethylformamide solution (porous membrane).

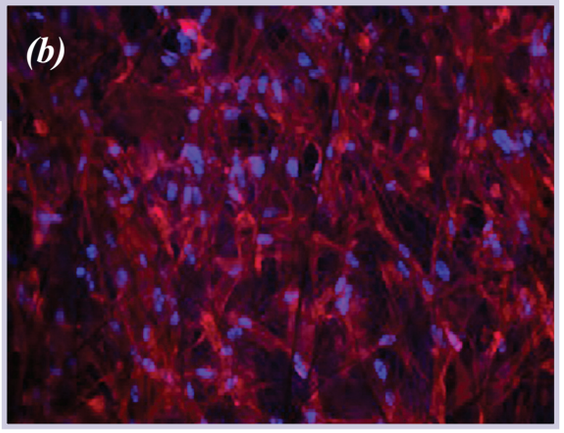
As shown, elecrospinated membranes analyzed by SEM have a fibrous morphology with fibers of about 1 micrometer.



SEM images of cellulose-dextran hydrogels with different compositions

Compatibility studies of the two developed hydrogels with human valvular cells showed an increased cell viability for both, with higher infiltration of human cells in electrospun membranes.





Microscopy images of
***(a)** cryogenated hydrogels or*
***(b)** electrospun membranes and populated with: (a) human fibroblast or (b)human valvular interstitial cells: green- calcein; red –Phalloidin; blue nuclei stained with DAPI.*

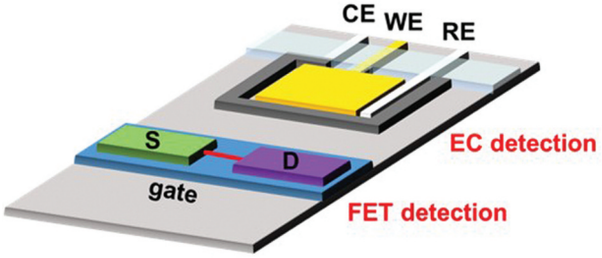
Ongoing experiments are designed to characterize the phenotype of cells cultivated on each hydrogel and to analyse the inflammatory profile on human valvular cells grown in 3D conditions compared with bi-dimensional culture.

4. ON-LINE MEASUREMENT OF LASER-DRIVEN PROTON BEAMS EFFECT ON HUMAN CELLS (ELI-RO project; ICBP coordinator: Elena Butoi; Project Coordinator: Adrian Enache INCDFM)

Investigation of the interaction between different kinds of radiation with biological materials has a great relevance in different fields such as aeronautics through improving radioprotection in space missions and radiobiology for treatment of various diseases especially cancer.The effect of radiation on biological systems involves multiple physical, chemical and biological steps. Direct effects results in a large number of reactive oxygen species (ROS) and reactive nitrogen species (RNS) within and outside cells, which are responsible for oxidative stress. Indirect effects are defined as alteration of normal biological processes and cellular components (DNA, protein, lipids, etc.).

The main goal of this research project is to develop of an on-line measurement system of laser-driven proton beams effect on epithelial and endothelial human cells. The real-time detection of ROS/RNS and intracellular chemical modification represent a major challenge.

Another important objective of the project is to identify the molecular markers related to oxidative stress response of cells exposed to proton irradiation.

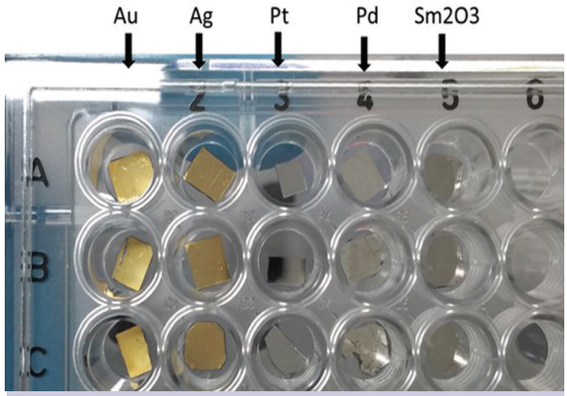


Schematic representation of the EC/ncFET detector. CE, WE, RE represents counter, working and reference electrodes. S and Dare the source and drain terminals.

RESULTS:

► Culture of endothelial cells on different metal films and estimation of cells viability

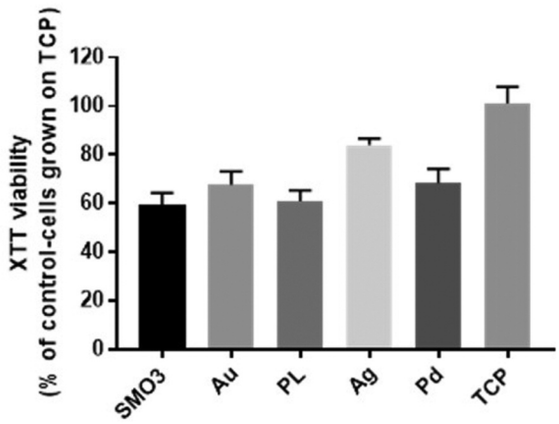
In an initial approach to analyze the effects of different metal films on endothelial cells viability in order to find the proper metal for detection electrode, we cultured human endothelial cells on the rectangular pieces of WE electrode covered with a thin film of Au, Ag, Pl, Pd, samarium oxideand on the TCP (tissue culture plates) wells (as positive control), and let them to grown for 72h. After this time, the 2,3-bis (2- methoxy -4- nitro-5 - sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay was performed to assess cell viability.



The experimental design of endothelial cells cultured on wells covered with different metal surfaces: gold (Au), silver (Ag), platinum (Pt), palladium (Pd) and Samarium oxide (Sm2O3).

According to the XTT results, endothelial cells grown on different substrates present different degrees of viability, with cells grown on Ag films exhibiting more than 80% of viability and on samarium oxide around 60% of control - cells grown on culture dish (TCP wells).

DEPARTMENT OF BIOPATHOLOGY AND THERAPY OF INFLAMMATION

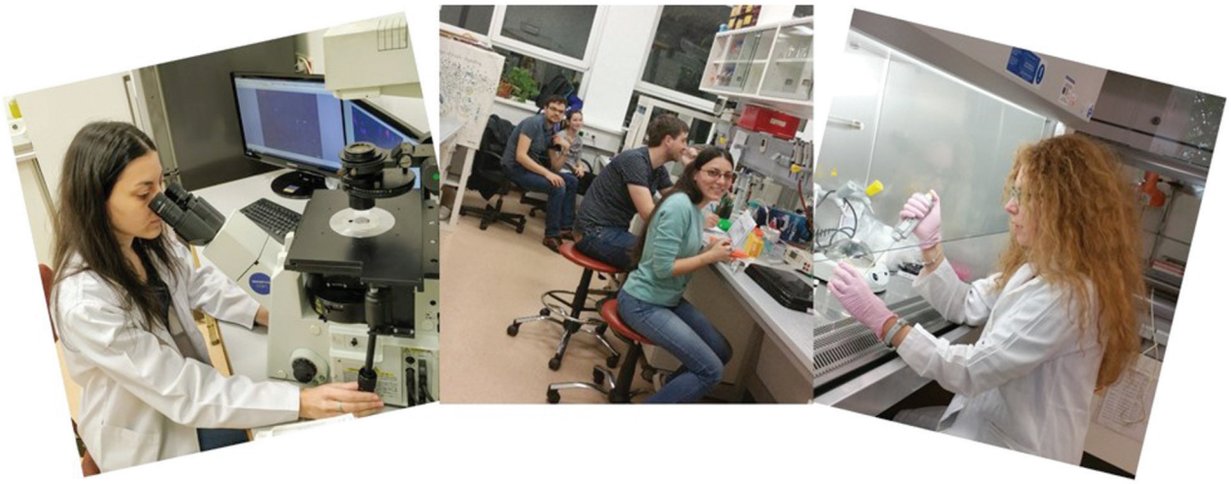


XTT assay results for endothelial cells EAhy926 grown on different metal films. Note that the silver and gold present the best viability of all investigated metals (70%, 80%, respectively from positive control, TCP).

Ongoing experiments are designed to investigate the impact of proton irradiation on oxidative stress in endothelial and epithelial cells. Identifying the sources of reactive oxygen species (ROS) and specific molecular markers which underlie the biological susceptibility of cells to the damaging effects of radiation will bring information about cell dysfunctions produced by ROS and will establish the safe radiation doses.

PERSPECTIVES

- To identify the specific mechanisms of aortic valve disease progression;
- To identify relevant and specific biomarkers for vascular inflammation associated with atherosclerosis and diabetes as targets for nanotherapy;



Manuela Călin, *PhD*

Head of Laboratory
E-mail:
manuela.calin@icbp.ro

MEDICAL AND PHARMACEUTICAL BIONANOTECHNOLOGIES LABORATORY

Major position/appointments

- Principal Investigator, Scientific Researcher grade I
- Member of the Scientific Councilof ICBP "N. Simionescu"
- Expert evaluator of the national grants
- Supervision of Graduate Students and Postdoctoral Fellows
- Invited Peer Reviewer for International Scientific Journals

STAFF

Daniela Rebleanu, *PhD /* ***Mihaela G. Cărnuță,*** *PhD /*
Florentina M. Safciuc, *PhD /* ***Cristina A. Constantinescu,*** *PhD student/*
Elena V. Fuior, *PhD student /* ***Geanina Voicu,*** *PhD student /*
Maria Anghelache, *Master student /* ***Marilena Misici,*** *Technical assistant*

SELECTED NEW FINDINGS OF THE LABORATORY

NANOTHERAPY

- VCAM-1 is an appropriate target for specific delivery of drugs to activated endothelial cells employing immunoliposomes

- Superoxide dismutase entrapped-liposomes restore the impaired endothelium-dependent relaxation of resistance arteries in experimental diabetes

- Endothelial VCAM-1 directed target-sensitive liposomes carrying CCR2 antagonists bind to activated endothelium, diminish adhesion and transmigration of monocytes, reduce the atherosclerotic lesions in ApoE-deficient mice and prevent the generation of pulmonary metastases in a murine and a human xenograft (patient-derived cells) model.

- Curcumin encapsulated in polymeric nanoparticles displays anti-inflammatory activity on TNF-α–activated endothelial cells by suppressing the phosphorylation of p38MAPK.

- Cell-penetrating peptides-functionalized curcumin-loaded lipid nanoemulsions are efficiently internalized by the endothelial cells, producing anti-inflammatory effects; when administrated intravenously in mice exhibit increased accumulation in the liver and the lungs.

- P-Selectin targeted dexamethasone-loaded lipid nanoemulsions reduce selectively the endothelium activation and the consequent monocyte infiltration and diminish significantly the lungs' inflammation, in a mouse model of acute inflammation.

- Lipopolysaccharide-induced inflammation in monocytes/macrophages is blocked by the liposomal delivery of Gi-protein inhibitor.

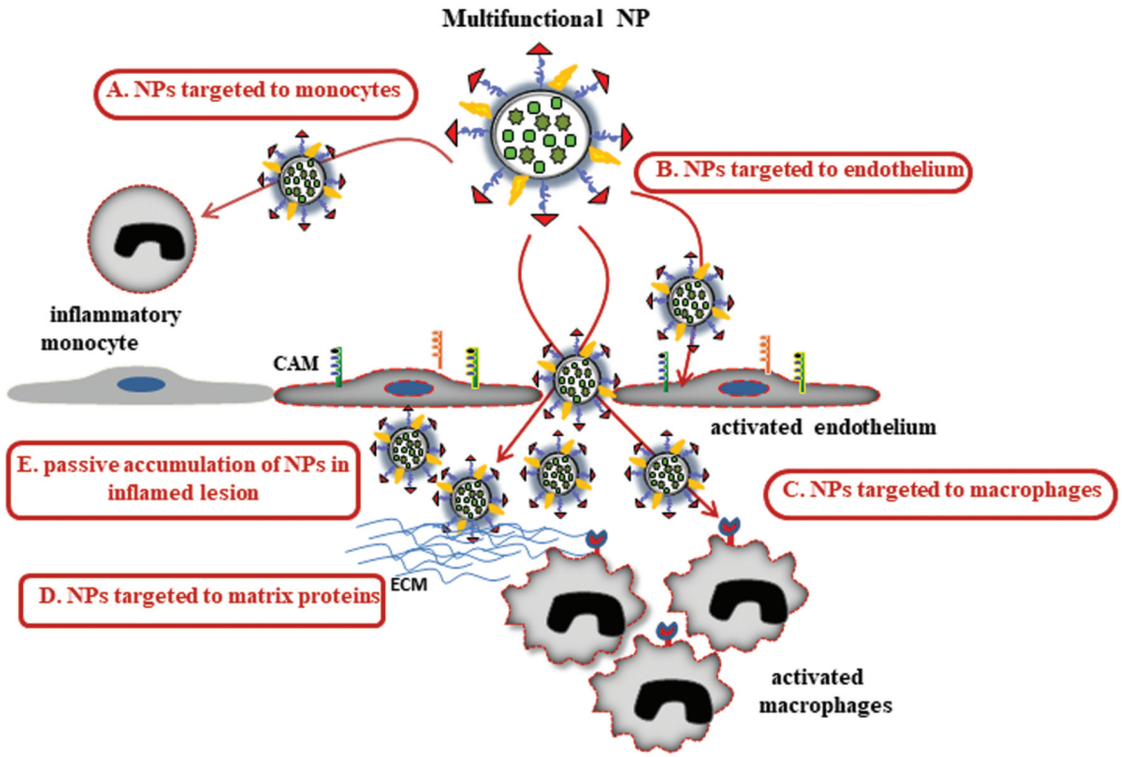
- P-selectin targeted PEGylated cationic liposomes bind specifically to activated endothelial cells and deliver with high-efficiency siRNA into the cells, that subsequently knock-down the mRNA expression of the target gene.

- VCAM-1 targeted lipid nanoemulsions deliver polyphenols to activated EC and have the functional capacity to lower monocyte infiltration by a mechanism involving the inhibition of NF-kB nuclear translocation and a reduced level of MCP-1 chemokine.

CURRENT PROJECTS

NANOTECHNOLOGY-BASED THERAPIES: A NEW PROSPECT FOR TREATMENT OF VASCULAR INFLAMMATION IN ATHEROSCLEROSIS

Recently, the emergence of nano-technology uses in medicine (i.e., nanomedicine) has opened a new prospect for the development of targeted therapies for atherosclerosis based on drug nanocarriers.Nanoparticles employed for biomedical applications typically have sizes below 100 nm and can be manufactured from a variety of organic materials (carbon, lipids, polymers), metallic or inorganic materials (gold, silver, or metal oxides), or hybrids of these materials. The development of different nanocarriers with tunable composition, architecture, and functionalities designed to improve diagnosis and clinical intervention in atherosclerosis has been boosted in the last few years.



Nanotechnology-based approaches envisaged to exploit the increased permeability at sites with vascular inflammation and the passive accumulation of nanoparticles in atheromatous lesions and also, the use of specific molecular targets exposed on surfaces of activated endothelium or monocytes/macrophages in vascular locations with plaques to diagnose and/or threat inflammatory atherosclerosis (*Calin M, Manduteanu I, Curr Med Chem. 2017;24(6):550-567).*

MEDICAL AND PHARMACEUTICAL BIONANOTECHNOLOGIES LABORATORY

1. DEVELOPMENT OF SUITABLE NANOCARRIERS TO PERFORM SPECIFIC AND EFFECTIVE DELIVERY OF THERAPEUTIC AGENTS TO DYSFUNCTIONAL ENDOTHELIAL CELLS

The endothelium-targeted therapeutic intervention has attracted a lot of interest and thereare hopes that this approach will lead to the progress in the treatment of many human pathologies having an inflammatory-associated process. In response to noxious stimuli, the affected endothelial cells (EC) become "activated" and overexpress cell adhesion molecules, chemokines, and cytokines that control the recruitment of circulating leukocytes into the vessels' intima leading thus to an inflammatory process. Thus, cell adhesion molecules that are overexpressed on the plasma membrane of activated EC can be used as molecular targets for nanotherapy.

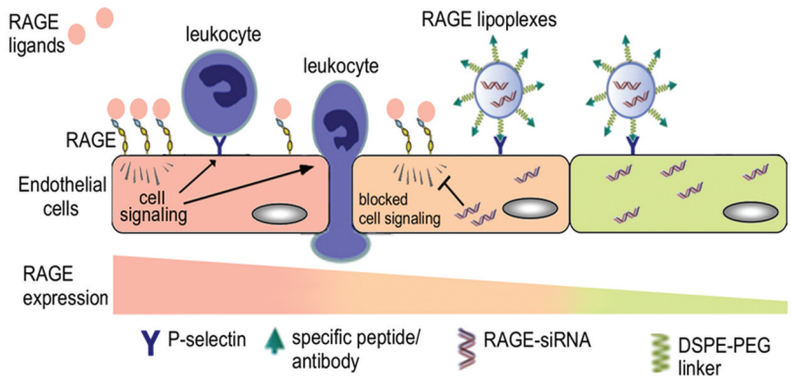
Our **goal** is to design different types of endothelium-targeted nanoparticles to achieve a vectorized delivery of therapeutic agents (e.g. small pharmacological compounds, siRNA/shRNA) to "inflamed" vascular endothelium.

OBJECTIVES:

- **Endothelial-targeted nanotherapy designed to silence receptor for advanced glycation end products (RAGE) and reduce inflammation in atherosclerosis**

Recent evidence shows that RAGE (Receptor for Advanced Glycation End products)initiates and perpetuates vascular inflammation process by promoting leukocyte infiltration into the vascular wall. In the recent years, RNA interference (RNAi) emerged as a powerful and widely used method for specific silencing the genes that contribute to the progression and exacerbation of chronic inflammation.

To decrease the inflammatory process that accompany the development of the atherosclerotic plaque we envisage a strategy to selectively deliver nanoparticles-loadedwith siRNA/shRNA sequences specific for RAGE at sites of activated endothelium.



Working hypothesis: *Endothelium-targeted nanoparticles (NPs) carrying RAGE-siRNA/shRNAare obtained by attaching to NPs' surface ligands which specifically recognize a particular molecule expressed mainly by activated endothelial surface (i.e.VCAM-1, P-selectin). The specific cellular delivery of the RAGE-siRNA/shRNA mediated by NPs into EC will reduce the expression of RAGE receptors on endothelium surface and thus will reduce endothelium inflammation (i.e. decreased activation of pro-inflammatory signalling pathways) and will interfere (diminish) leukocyte recruitment into the plaque.*

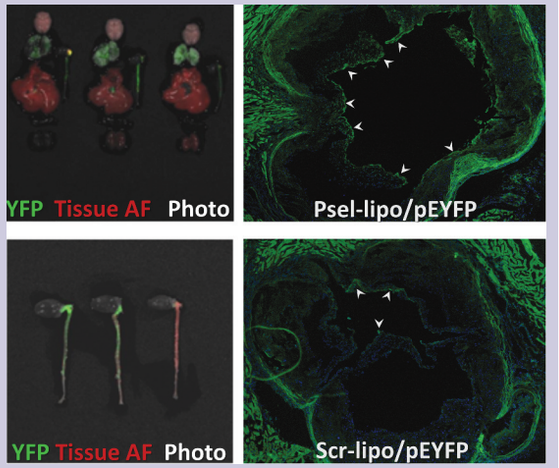
The cell adhesion molecule, P-selectin can be used as target for nanocarriers because of its strong presence on the membrane of activated EC in both acute and chronic inflammation. To endow specificity for activated EC, a peptide with affinity for P-selectin was covalently coupled to the distal ends of PEGylated phospholipid anchor inserted in the membrane of liposomes (Psel-lipo).

RESULTS:

►Development of Psel-lipo/siRNA nanocarriers, that are able to efficiently protect the encapsulated siRNA from exogenous factors.

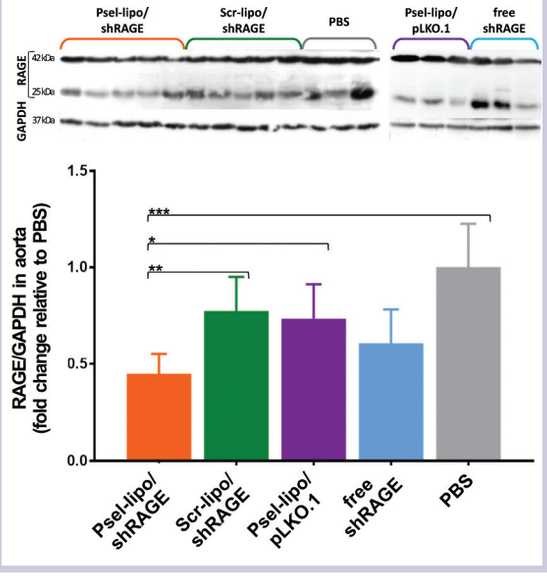
These targeted siRNA nanocarriers bind specifically to TNF-α activated endothelial cells, deliver with high efficiency siRNA into the target cells thus leading to specific silencing of the chosen gene (*Constantinescu CA et al., Pharmaceutics, 2019)*.

►P-selectin targeted nanocarriers bind specifically to the aorta of Apo E-deficient mice, and the administration of lipoplexes made of Psel-lipo and a plasmid pEYFP, encoding YFP (yellow fluorescent protein), determined at 48 hours the expression of YFP in the aorta and aortic valve leaflets of ApoE-deficient mice.



In vivo targeted delivery of pEYFP by Psel-lipo/pEYFP lipoplexes. Spectral unmixing images show YFP expression (green) and tissue autofluorescence (red) in organs (A) and aorta (B) of ApoE-deficient mice treated with Psel-lipo/pEYFP (1,2) and non-targeted Scr-lipo/pEYFP (3) lipoplexes; The expression of YFP in the endothelium covering the aortic valve leaflets in ApoE-deficient mice treated with Psel-lipo/pEYFP (C) or Scr-lipo/pEYFP (D) lipoplexes.

►The administration of Psel-lipo/shRNA-RAGE lipoplexes in ApoE-deficient mice is effective in downregulating the expression of RAGE in the aorta.



*Western blot analysis demonstrated that expression of RAGE (both isoforms) in the aorta was significantly diminished after treatment with Psel-lipo/shRAGE for four weeks (2 injections/week) compared with controls: Scr-lipo/shRAGE, Psel-lipo/pLKO.1, PBS and free shRNA-RAGE. *p<0.05, **p<0.01, ***p<0.001.*

Ongoing experiments are designed to investigate the impact of treatment with Psel-lipo/shRAGE on the development of the atherosclerotic lesion. Moreover, the toxicity

and immune-safety of the in vivo administration of this nanocarrier to be used for specific silencing the genes that contribute to the progression and exacerbation of chronic inflammation will be evaluated.

● Lipid-based nanoparticles designed to function as vectors for targeted delivery of bioactive compounds in vascular inflammation therapy

Polyphenols represent a large class of compounds, occurring as secondary metabolites in plants, with numerous therapeutic effects, such as anti-inflammatory and anti-oxidant properties, anti-microbial, anti-tumoral, anti-angiogenic and immunomodulatory activity. However, all these beneficial effects are hindered by their poor water solubility and low bioavailability.

We hypothesize that the encapsulation of polyphenols into lipid nanoemulsions (LN) may overcome these limitations and that the targeting of these polyphenol-loaded LN to vascular cell adhesion molecule 1 (VCAM-1), highly expressed on activated EC, could reduce EC inflammation.

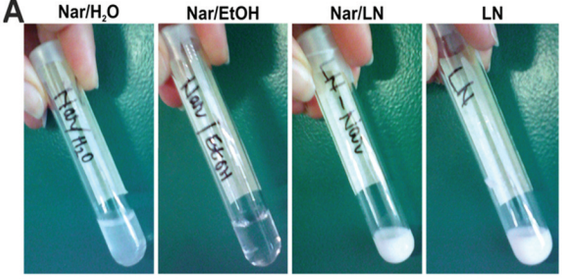
RESULTS:

► Endothelium-targeted flavonoids-loaded lipid nanoemulsions exert anti-inflammatory effects on activated endothelial cells.

We successfully incorporated naringenin and hesperetin, two flavonoids with poor hydrosolubility into lipid nanoemulsions targeted to VCAM-1. These nanoemulsions displayed good in vitro stability, and slow release of the cargo. Furthermore, they did not exhibit *in vitro* cytotoxicity as assessed on the EC line EA.hy926, nor did they provoke lysis of mouse erythrocytes. The flavonoid-loaded LN exerted anti inflammatory effects as supported by functional monocyte adhesion and trans-migration assays and reduced the expression of the pro-inflammatory molecule MCP-1 and the nuclear translocation of NF-kB.

The data indicate that the beneficial effect of flavonoids can be significantly improved by protecting them via encapsulation in LN and suggest their potential therapeutic use in reducing the endothelial inflammation at the inception of atherosclerotic process.

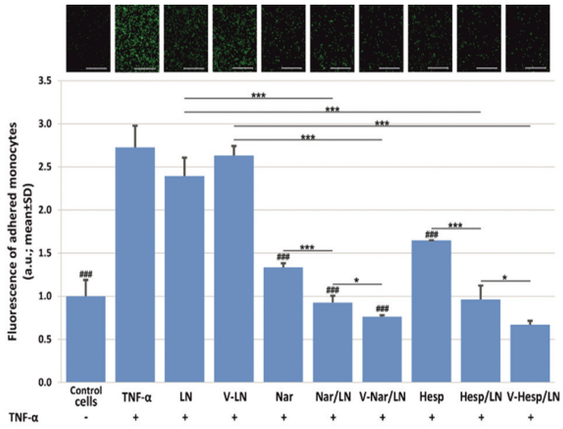
A



B

LN type	Flavonoid	Targeting peptide	Dimension (intensity distribution)		Zeta potential (mV)
			Z-average (nm)	PDI	
LN	-	-	210.4 ± 0.655	0.211 ± 0.009	- 24.7 ± 1.80
V-LN	-	+	188.8 ± 2.228	0.211 ± 0.014	- 49.7 ± 1.25
Nar	naringenin	-	190.4 ± 2.272	0.191 ± 0.010	- 34.4 ± 0.32
V-Nar	naringenin	+	200.3 ± 0.264	0.219 ± 0.013	- 61.2 ± 2.00
Hesp	hesperetin	-	193.2 ± 0.472	0.201 ± 0.018	- 34.2 ± 0.92
V-Hesp	hesperetin	+	219.7 ± 5.667	0.242 ± 0.028	- 58.3 ± 0.67

*(A) Photographs depicting naringenin dispersed in water, dissolved in ethanol or encapsulated into lipid nanoemulsions. (**B**) Average hydrodynamic diameter and zeta potential of empty and flavonoid-loaded nanoparticles (non-targeted and VCAM-1 targeted).*



*VCAM-1 targeted flavonoid-loaded nanoemulsions are functional in the reduction of monocyte adhesion to TNF-α activated EC compared with the same concentration of free flavonoids (50 μM). *, p<0.05; **, p<0.01; ***, p<0.005.*

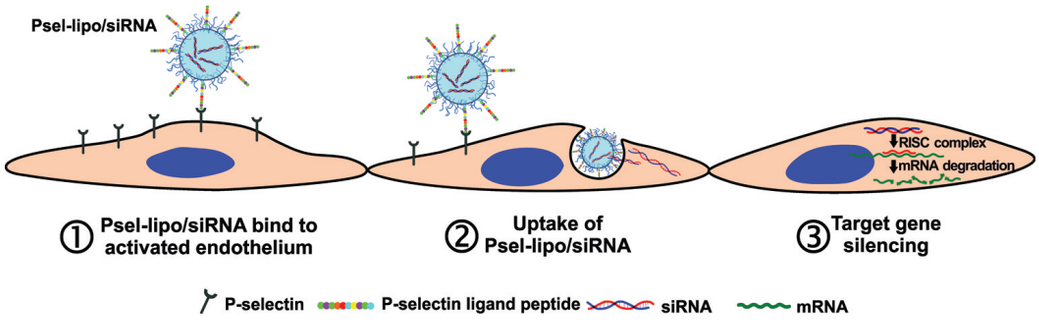
Ongoing experiments are focused on the investigation of the *in vivo* therapeutic effects of endothelium-targeted polyphenols-loaded LN in appropriate pre-clinical animal models.

2. DEVELOPMENT OF NANO-THERAPEUTICS FOR TARGETED DELIVERY OF SIRNA/SHRNA TO AORTIC VALVE TO DOWN-REGULATE GENES THAT DRIVES AORTIC VALVE DISEASE

Recent data suggest that in the early phase of aortic valve disease (AVD), inflammation can determine a subset of aortic valvular endothelial cells (VEC) to undergo endothelial to mesenchymal transformation (EndMT) and also, can initiate the osteodifferentiation of valvular interstitial cells (VIC) that actively contribute to aortic valve calcification.

We hypothesize that the blocking of the two key features of aortic valve lesions, namely EndMT and VIC's osteodifferentiation using nanocarriers of siRNA/shRNA to downregulate genes that drive EndMT (e.g. SMAD3, SNAI1) and VIC osteodifferentiation (e.g. RUNX2)may represent a therapeutic strategy with an impact for early stages of AVD.

The **objective** is to design effective nanocarriers of siRNA/shRNA able to target valvular cells or extracellular matrix in diabetes-affected aortic valve and to block EndMT and VIC to osteoblasts transformation.



Targeted delivery of siRNA/shRNA to valvular cells using nanocarriers.

RESULTS:

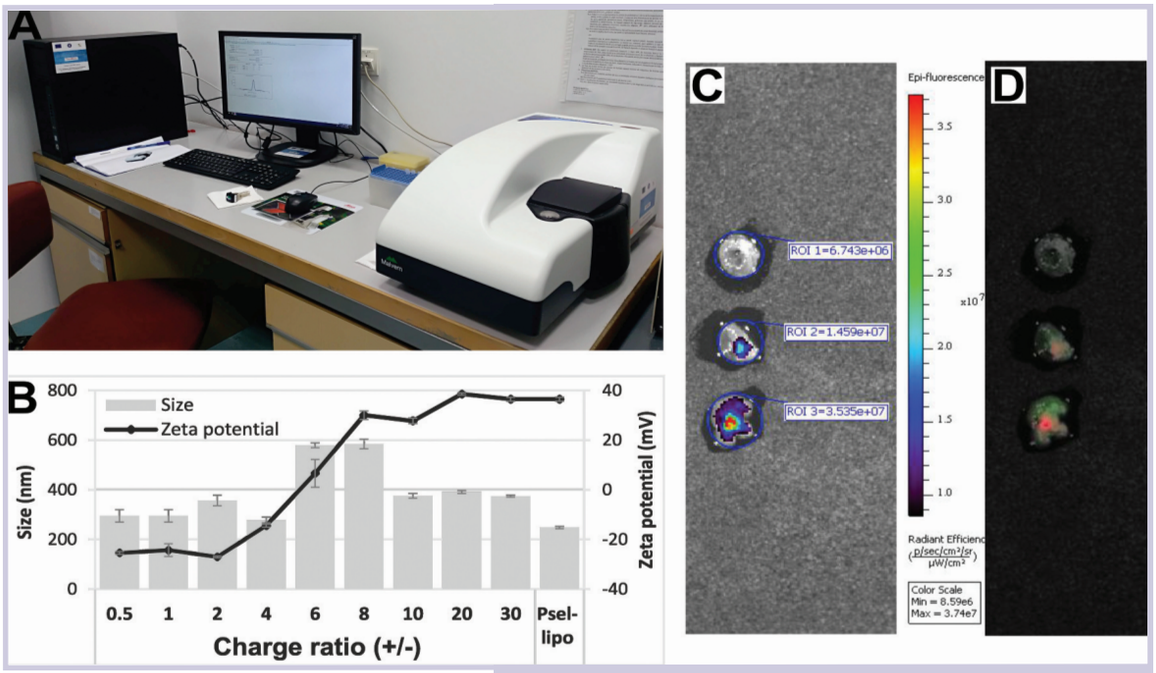
► **Design, preparation and characterization of siRNA / shRNA-carrying nanoparticles (siRNA/ shRNA-NPs) targeted to P-selectin expressed on valvular endothelial cells surface (VEC)** (patent application OSIM nr. A/00811, *Constantinescu CA et al., Pharmaceutics, 2019)*. These P-selectin targeted siRNA / shRNA nanocarriers bind to cultured EC and aortic valve of ApoE-deficient mice.

Ongoing experiments are designed to optimize nanocarriers targeted to P-selectin or other molecules, such as VCAM-1 or collagen IV to obtain maximal transfection efficiency of siRNA/shRNA in VEC and VIC exposed to high glucose conditions in the presence or absence of osteogenic factors. Specific shRNA sequences for relevant moleculesinvolved in EndMT and VIC osteodifferentiation are envisaged for the evaluation of the *in vitro* and *in vivo*therapeutic effects of targeted nanocarriers.

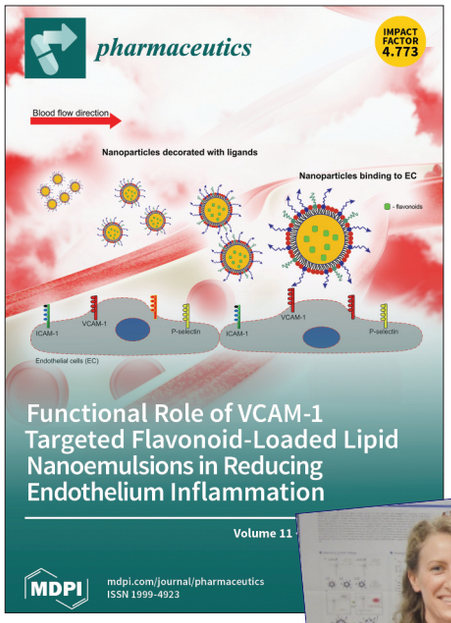
PERSPECTIVES

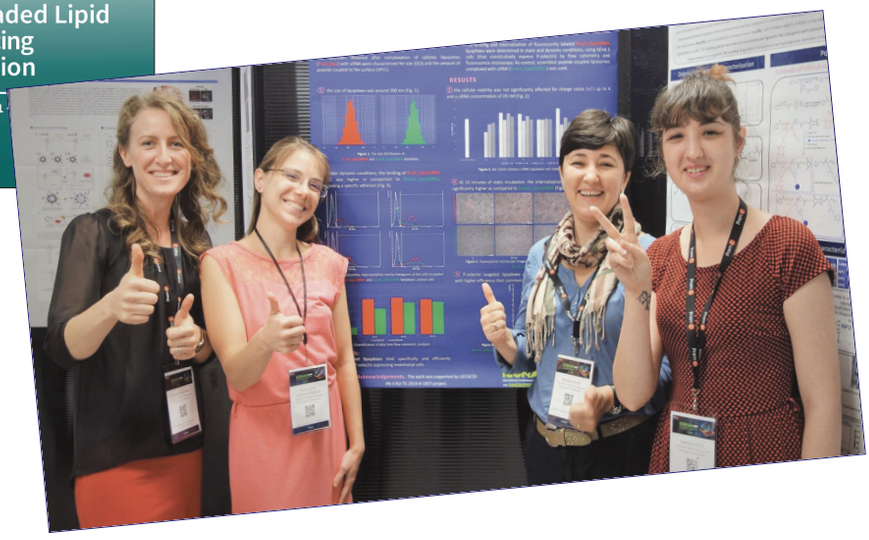
- Design of novel nanoparticle-based drug delivery systems to specifically target inflamed endothelium and monocytes/macrophages and to modulate their affected functions;

- The development of new targeted nanotherapies for aortic valve disease.



*(**A**) Zetasizer Nano ZS (Malvern Instruments, UK) used for (**B**) size and zeta potential measurements of siRNA/shRNA nanocarriers; (**C**) The accumulation of fluorescently labelled P-selectin targeted lipoplexes (c) and non-targeted lipoplexes coupled with a scrambled peptide (b) to the aortic valve of ApoE-deficient mice. Control aortic valve from a mouse injected with PBS (a); (**D**) Spectral unmixing showing the autofluorescence of the tissue (green) and the specific accumulation of P-selectin targeted fluorescently-labelled lipoplexes (red) in the aortic valve of ApoE-deficient mouse.*





COLLABORATION OF THE DEPARTMENT

INTERNATIONAL

- Cardiovascular Tissue Engineering and Regenerative Medicine Lab, Clemson University, USA (Dan Simionescu)
- Biocompatibility and Tissue Regeneration Lab, Clemson University, USA (Agneta Simionescu)
- Department of Clinical Sciences, Malmö, Lund University, Sweden (Alexandru Schiopu)
- Department of Kardiovaskuläre Molekularbiologie, Universitätsklinikum Aachen, Germany (Christian Weber, Rory Koenen)
- Department of Pharmacy, Martin-Luther University, Halle, Germany (Gerd Bendas)
- Department of Pharmacy, University of Bonn, Germany (Gerd Bendas, Martin Schlesinger)
- Faculty of Pharmacy, University of Istanbul, Turkey (Erdal Cevher)
- Institute of Physiology, University of Zürich, Switzerland (Lubor Borsig, Marko Roblek)
- University of Minho, Portugal (Sandra Carvahlo)
- Montana University of Leoben, Austria (Robert Franz)

NATIONAL

- Institute of Cardiovascular Disease "Prof. Dr. C.C. Iliescu",Bucharest (Carmen Ginghina)
- Emergency Hospital of M.A.I. Prof. Dr. D. Gerota, Bucharest, Romania (Monica Căpraru)
- University of Medicine and Pharmacy" Carol Davila", Bucharest, România (Dragoș Vinereanu)
- Center of Surface Science and Nanotechnology, University Politehnica of Bucharest (Marius Enăchescu)
- Centrul Clinic de Urgenţă de BoliCardiovasculare"Dr. Constantin Zamfir" (Ionel Droc)
- The Institute for Research and Development of Textiles and Leather, Bucharest (Carmen Gaidau)
- Institute of Macromolecular Chemistry "Petru Poni, Iași (Mariana Pinteală)
- National Institute of Materials Physics (Ionuț Enculescu)
- Department of Natural and Synthetic Polymers, "Gh. Asachi" Technical University of Iași (Geta David)
- SC Optoelectronica 2000 SRL, Magurele (Teodor Necșoiu, Mihai Șerbănescu)

MEDICAL AND PHARMACEUTICAL BIONANOTECHNOLOGIES LABORATORY

GRANTS AWARDED BY COMPETITION (1999-2019)

● **2018-2022**: **PCCF project, cod PN-III-P4-ID-PCCF-2016-0172 (INNATE-MI)**: "*Targeting innate immune mechanisms to improve risk stratification and to identify future therapeutic options in myocardial infarction*", project coordinator: Acad. Maya Simionescu - IBPC "N. Simionescu. Partners: University of Medicine, Pharmacy, Science and Technology of Târgu Mureș, "CAROL DAVILA" University of Medicine and Pharmacy Bucharest.

● **2018-2022**: **PCCF project, cod PN-III-P4-ID-PCCF-2016-0050 (5D-nanoP),** *"Mimicking living matter mechanisms by five-dimensional chemistry approaches"* partners:Institute of Macro-molecular Chemistry "Petru Poni, Iași; IBPC "N. Simionescu", Bucharest; Center for Organic Chemistry "Costin D. Neniţescu", Bucharest (partner responsible: Maya Simionescu and Manuela Călin).

● **2018-2020**: **PCCDI Complex Project nr. 13 PCCDI/2018(INTERA)** *"Intelligent therapies for non-communicable diseases based on controlled release of pharma-cological compounds from encapsulated engineered cells and targeted bionanoparticles*" (complex project coordinator: Maya Simionescu), INTERA2 component project: "Development of a 3D platform designed for pre-clinical drug testing composed of cells incorporated into three-dimensional bio-matrices", (project INTERA2 coordinator: Elena Butoi); INTERA3 component project:" Intelligent *nanobioparticles designed to function as vectors for targeted delivery of bioactive compounds in vascular inflammation therapy"* (project INTERA3 coordinator: Manuela Călin).

● **2014-2020**: **Competitiveness Operational Programme Project, Priority Axis1/Action 1.1.4 (THERAVALDIS)**: *"Targeted therapies for diabetes -related aortic valve disease",*Financing Contract no.115/13.09.2016/ MySMIS: 104362 - executive manager: Ileana Mânduțeanu.

● **2017-2019**: **ELI-RO/ PN-III-P5-Subprogramme 5.1**, *On-line measurement of laser-driven proton beams effect on human cells*. Project coordinator Adrian Enache- National Institute of Materials Physics, (ICBP- N Simionescu, partner responsible: Elena Butoi).

● **2015-2018**: **SIINN ERA-NET project FP7 scheme (NANO_SAFE_LEATHER):** "*The effect on human health of Ag/TiO2 NM-treated leathers for footwear industry*", partners: The Institute for Research and Development of Textiles and Leather, Romania; ICBP "Nicolae Simionescu", România; University of Minho, Portugal; Montana University of Leoben, Austria; SC TARO COMMIMPEX LTD, România (partner responsible: Manuela Călin).

● **2015-2017**: **Grant PN-II-RU-TE-2014-4-0965**, "*MicroRNA signature of vascular cells cross-talk relevant for the atherosclerotic plaque rupture in patients with type II diabetes*" (project coordinator: Elena Butoi).

● **2015-2017**: **PNCDI II Grant nr. PN-II-RU-TE-2014-4-1837 (**funded by UEFISCDI): *"Endothelium-targeted Nano-therapies designed to silence receptor for advanced glycation products (RAGE) in atherosclerosis",* (project coordinator: Manuela Călin).

● **2011-2014**: **EuroNanoMed ERA-NET project FP7 scheme (NANODIATER)** "*Nanoparticles designed to target chemokine-related inflammatory processes in vascular diseases and cancer metastasis and implementation of a biosensor to diagnose these disorders*", partners: ICBP "Nicolae Simionescu", Romania; Center of Surface Science and Nanotechnology, University Polyethnic of Bucharest; University of Bonn, Germany; Istanbul University, Turkey; University of Zürich, Switzerland;EPO Berlin GmbH , Germany; SC Optoelectronica 2000 SRL (project director: Maya Simionescu; Research & Development coordinator: Manuela Călin).

● **2011-2014: Project PNII: IDEAS**: "*Molecules and mechanisms involved in vascular inflammation dependent on cytokines and chemokines as possible targets for new*

nanoterapeutic strategies", (project coordinator: Ileana Mânduțeanu).

• **2007-2010**: **Project PNCDI2- P4 project: "***Molecular links between chronic inflammation and accelerated atherosclerosis:role of resistin and the newly identified chemokines:fractalkine and CXCL16:new avenues for targeted therapies"*, (project coordinator: Ileana Mânduțeanu).

• **2006-2008**: **National Authority for Scientific Research and Innovation (ANCS) Grant** nr.1423/2006: "*Study of signaling pathways involved in hyperglycemia - induced fractalkine expression and their targeting, a new approach to the therapy of cardiovascular pathologies associated with diabetes"* (project coordinator: Manuela Călin)

• **2004-2006**: **VIASAN PNCDI Grant**, nr. 330/2004: *"A new strategy to stabilize the atherosclerotic lesions in acute coronary syndromes: suppression of activated macrophages using clodronate-loaded liposomes"*(project coordinator: Manuela Călin)

• **2005-2008**: **FP6 International project, Specific Support Action (SERA)**, Member in the management cometee, WP Leader Ileana Mânduțeanu.

• **2003-2005**: **Grant VIASAN, MEC**: "*The chemokine modulation in different vascular pathologies; their functional role*", (project coordinator: Elena Butoi).

• **2001-2003**: **VIASAN PNCDI Grant**, nr. 031/2001: *"Targeted delivery of drugs to activated endothelium using "smart" liposomes: a strategy for cardiovascular diseases therapy"* (project coordinator: Manuela Călin).

• **2003-2004**: **Romanian Ministry of Education and Research Grant:** *"Protective effects of aspirin in diabetes mellitus model, in vitro"* (project coordinator: Elena Butoi).

• **2003-2004**: **Romanian Academy Grant**: "*Study of the effect of superoxide dismutase administered in liposomes on the reactivity of mesenteric arteries isolated from diabetic hamsters"*(project coordinator: Manuela Călin).

• **2001-2002**: **Romanian Ministry of Education and Research Grant:** "*The effect of the anti-inflammatory drugs on the activated vascular endothelium*" (project coordinator: Elena Butoi).

• **1999-2001**: **National Agency for Science, Technology and Innovation (ANSTI) Grant** nr.5243/1999: "*Specific drug delivery to vascular endothelium with liposomes"* (project coordinator: Manuela Călin).

• **2000**: **Romanian Academy Grant**: "*Liposome characterization for drugs delivery"*. (project coordinator: Elena Butoi).

• **1999-2001**: **National Agency for Science, Technology and Innovation (ANSTI) Grant: "***The expression of cell adhesion molecules in valvular endothelium. Involvement in the future treatement of valvular diseases"*(project coordinator: Ileana Mânduțeanu).

• **1999**: **Romanian Academy Grant**, 376/1999: *"Interaction of liposomes with vascular endothelium"* (project coordinator: Manuela Călin).

• **1997-1998**: **Ministry of research and Technology Grant:**"*Mechanisms involved in monocyte adhesion to valvular endothelial cells grown in high glucose concentrations*" (project coordinator:Ileana Mânduțeanu).

• **1997**: **Romanian Academy Grant**: *"Monocyte adhesion to valvular endothelial cells grown in high glucose conditions"*(project coordinator:Ileana Mânduțeanu).

AWARDS

● **For Women in Science - L'Oréal-UNESCO Award,** 2019 (Monica Țucureanu)

● **Innovation Award and Gold Diploma** at "*EuroInvent 11th Edition European Exhibition of Creativity and Innovation 2019*"(Iași) and **Excellence Award at PRO INVENT, 2019** (Cluj Napoca) for invention: „*Vertical (electro) magnetic separator of isomagnetic nanoparticles*. **Inventors**: Denisa Ficai, Ioana Ardelelean, Cornelia Ilie, Manuela Călin, Elena-Veleria Fuior, Adrian Fifere, Mariana Pinteală, Gheorghe Fundueanu-Constantin, Anton Ficai, Maya Simionescu, Ecaterina Andronescu.

● **Herbert-Berler Prize of Excellence of the** Romanian Academy of Medical Sciences, 2015 [Manuela (Călin) Voinea]

● **Romanian Academy Prize in biology "Nicolae Simionescu"** for the series of papers published on the use of targeted nanotherapies for inflammation, 2015 [Manuela (Călin) Voinea and Elena (Butoi) Dragomir]

● **Constantin Velican Award** of the Romanian Society for Cell Biology, 2012 [Manuela (Călin) Voinea and Elena (Butoi) Dragomir]

● **For Women in Science - L'Oréal-UNESCO Award,** 2011 [Elena (Butoi) Dragomir]

● **First prize** awarded by the Romanian Society of Cell Biology, 2011 [Manuela (Călin) Voinea]

● **Prize for Excellence** awarded by the Romanian Medical Association, 2010 [Manuela (Călin) Voinea]

● **First prize** at the National Symposia VIASAN-CEEX, 2008 [Elena (Butoi) Dragomir]

● **Prize for Excellence** awarded by the Romanian Society of Cell Biology, 2003 [Manuela (Călin) Voinea]

● **Agora Diabetologica Prize**, of the XXVII National Congress for Diabetes, Nutrition and Metabolic Diseases, 2002 [Elena (Butoi) Dragomir]

● **European Life Scientist Organization Prize**, 2002 [Manuela (Călin) Voinea]

● **Bio-Rad Laboratories Prize** for valuable research with Bio-Rad equipment, 2002 (Ileana Mânduțeanu)

● **Daniel Danielopolu Prize** of the Romanian Academy, 1994 (Cristina Lupu, Maria Calb)

● **Emil Racoviță Prize** of the Romanian Academy, 1993 (Ileana Mânduțeanu)

DEPARTMENT OF BIOPATHOLOGY AND THERAPY OF INFLAMMATION