

## DEPARTMENT OF BIOPATHOLOGY AND THERAPY OF INFLAMMATION

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.

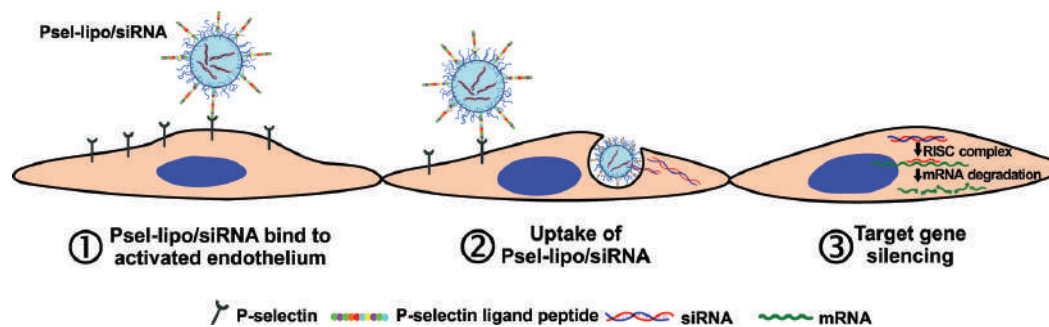
### 2. DEVELOPMENT OF NANO-THERAPEUTICS FOR TARGETED DELIVERY OF siRNA/shRNA TO AORTIC VALVE TO DOWN-REGULATE GENES THAT DRIVES AORTIC VALVE DISEASE

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

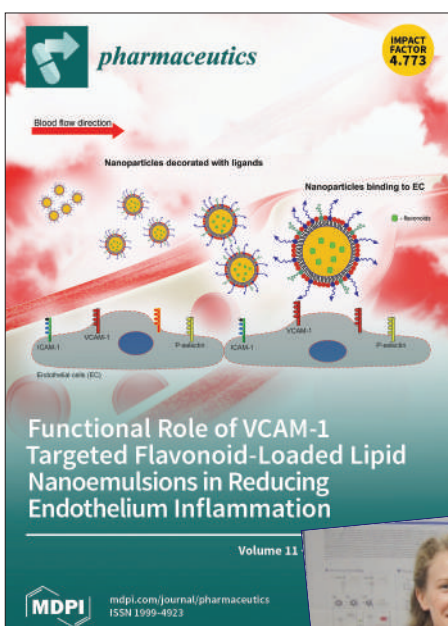
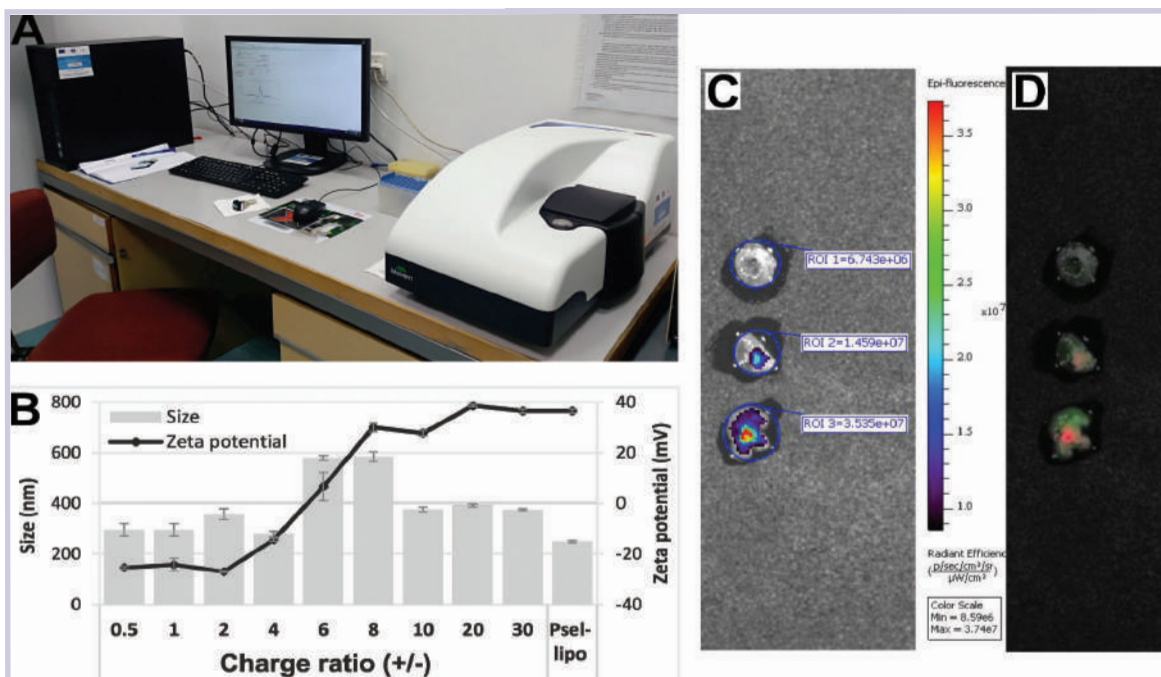
exposed to high glucose conditions in the presence or absence of osteogenic factors. Specific shRNA sequences for relevant molecules involved in EndMT and VIC osteodifferentiation are envisaged for the evaluation of the *in vitro* and *in vivo* therapeutic effects of targeted nanocarriers.

### PERSPECTIVES

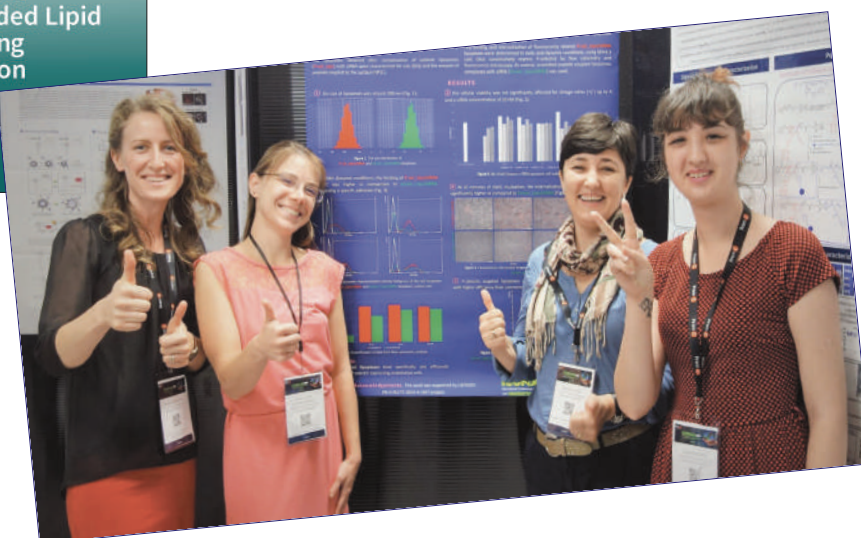
- Design of novel nanoparticle-based drug delivery systems to specifically target inflamed endothelium and monocytes/macrophages and to modulate their affected functions;

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

# MEDICAL AND PHARMACEUTICAL BIONANOTECHNOLOGIES LABORATORY



(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.





# DEPARTMENT OF BIOPATHOLOGY AND THERAPY OF INFLAMMATION

## 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 Urgență de Boli Cardiovasculare”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. Nenitescu”, 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*



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*nanotherapeutic 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 cometeet, 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).

# MEDICAL AND PHARMACEUTICAL BIONANOTECHNOLOGIES LABORATORY

## AWARDS

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

- **Innovation Award and Gold Diploma** at “EuroInvent 11<sup>th</sup> 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)



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