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## MEDICAL AND PHARMACEUTICAL BIONANOTECHNOLOGIES LABORATORY

### *Major position/appointments*

- Principal Investigator, Scientific Researcher grade I
- Member of the Scientific Council of 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. Cărnăuță, 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- $\alpha$ -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- $\kappa$ B nuclear translocation and a reduced level of MCP-1 chemokine.

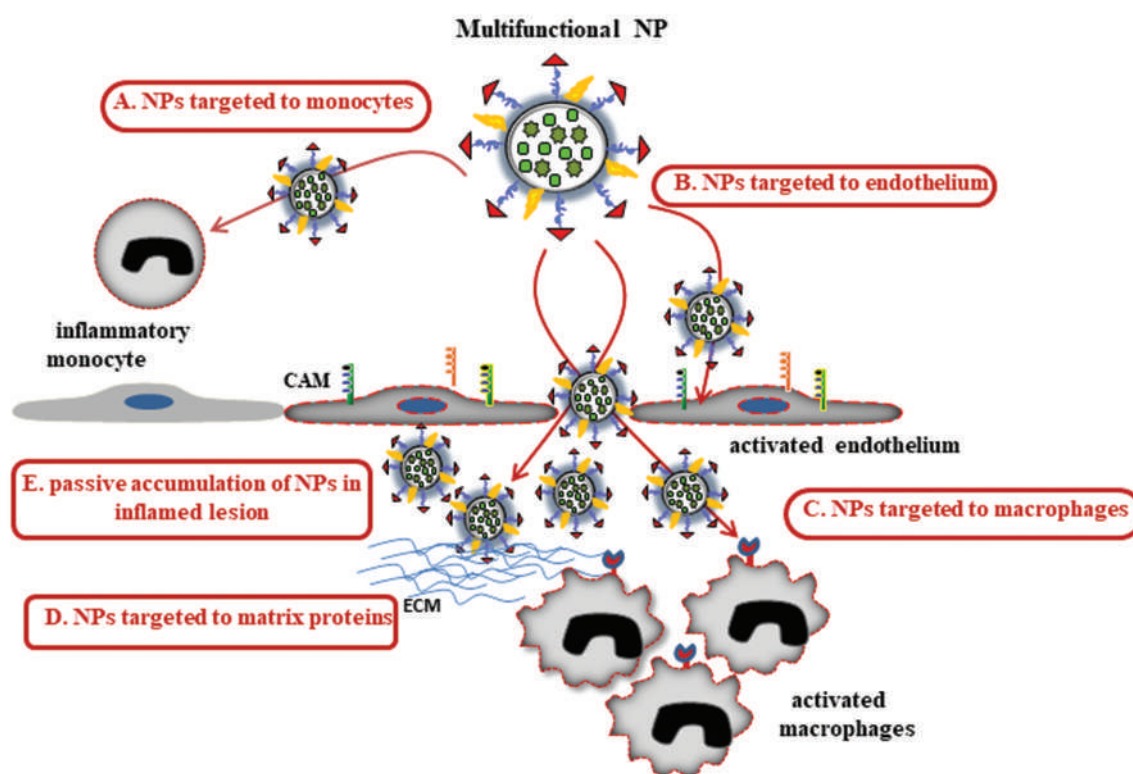
# DEPARTMENT OF BIOPATHOLOGY AND THERAPY OF INFLAMMATION

## 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 treat inflammatory atherosclerosis (Calin M, Manduteanu I, Curr Med Chem. 2017;24(6):550-567).*



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## 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 there are 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.

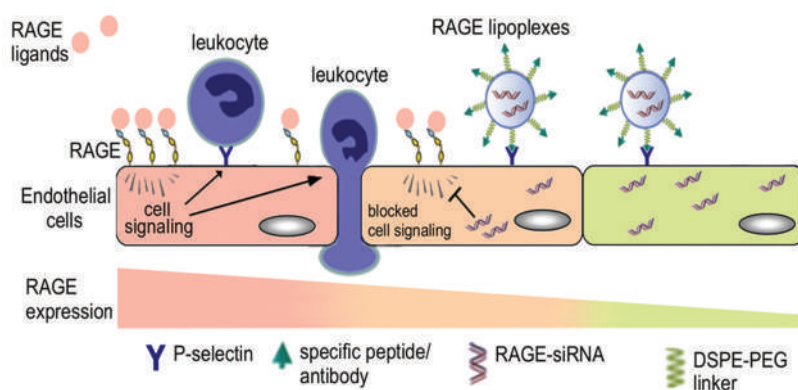
Our **goal** is to design different types of endothelium-targeted nanoparticles to achieve a vectorized delivery of therapeutic agents (e.g. small pharmacological compounds, siRNA/shRNA) to “inflamed” vascular endothelium.

### 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 loaded with siRNA/shRNA sequences specific for RAGE at sites of activated endothelium.



**Working hypothesis:** Endothelium-targeted nanoparticles (NPs) carrying RAGE-siRNA/shRNA are 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.

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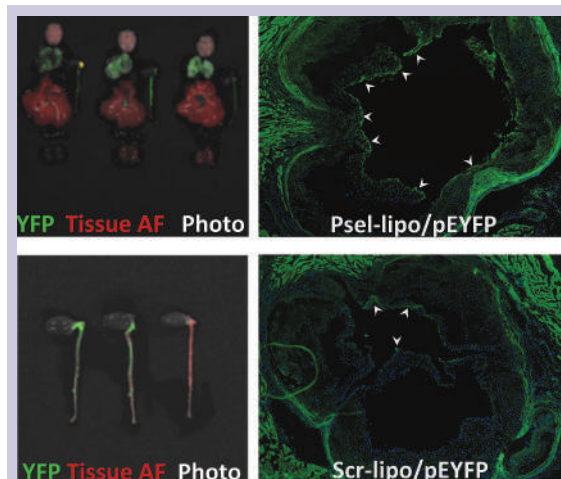
The cell adhesion molecule, P-selectin can be used as target for nanocarriers because of its strong presence on the membrane of activated EC in both acute and chronic inflammation. To endow specificity for activated EC, a peptide with affinity for P-selectin was covalently coupled to the distal ends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

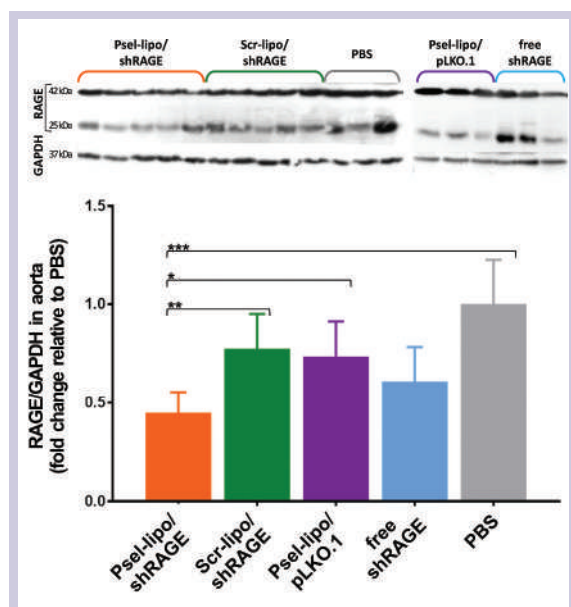
These targeted siRNA nanocarriers bind specifically to TNF- $\alpha$  activated endothelial cells, deliver with high efficiency siRNA into the target cells thus leading to specific silencing of the chosen gene (Constantinescu CA et al., *Pharmaceutics*, 2019).

► **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



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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.

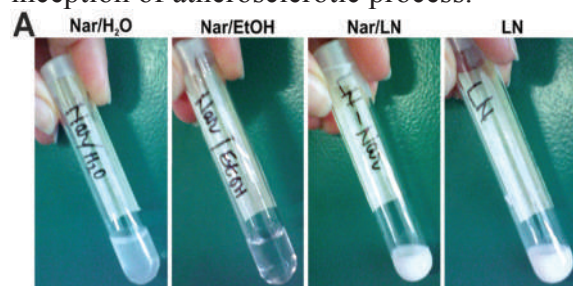
We hypothesize that the encapsulation of polyphenols into lipid nanoemulsions (LN) may overcome these limitations and that the targeting of these polyphenol-loaded LN to vascular cell adhesion molecule 1 (VCAM-1), highly expressed on activated EC, could reduce EC inflammation.

## 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 transmigration assays and reduced the expression of the pro-inflammatory molecule MCP-1 and the nuclear translocation of NF- $\kappa$ B.

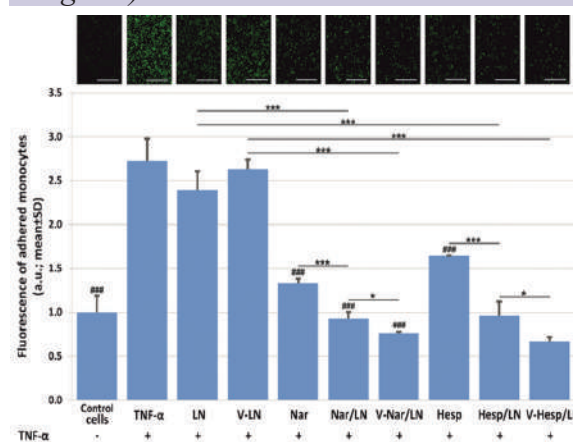
The data indicate that the beneficial effect of flavonoids can be significantly improved by protecting them via encapsulation in LN and suggest their potential therapeutic use in reducing the endothelial inflammation at the inception of atherosclerotic process.



**B**

LN type	Flavonoid	Targeting peptide	Dimension (intensity distribution)		Zeta potential (mV)
			Z-average (nm)	PDI	
LN	-	-	210.4 $\pm$ 0.655	0.211 $\pm$ 0.009	-24.7 $\pm$ 1.80
V-LN	-	+	188.8 $\pm$ 2.228	0.211 $\pm$ 0.014	-49.7 $\pm$ 1.25
Nar	naringenin	-	190.4 $\pm$ 2.272	0.191 $\pm$ 0.010	-34.4 $\pm$ 0.32
V-Nar	naringenin	+	200.3 $\pm$ 0.264	0.219 $\pm$ 0.013	-61.2 $\pm$ 2.00
Hesp	hesperetin	-	193.2 $\pm$ 0.472	0.201 $\pm$ 0.018	-34.2 $\pm$ 0.92
V-Hesp	hesperetin	+	219.7 $\pm$ 5.667	0.242 $\pm$ 0.028	-58.3 $\pm$ 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- $\alpha$  activated EC compared with the same concentration of free flavonoids (50  $\mu$ M). \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.005$ .