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:

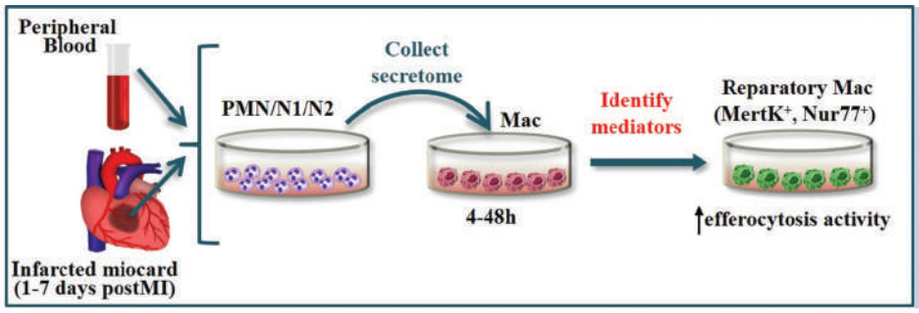
I) isolation of mouse and human polymorphonuclear neutrophils (PMN)

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.

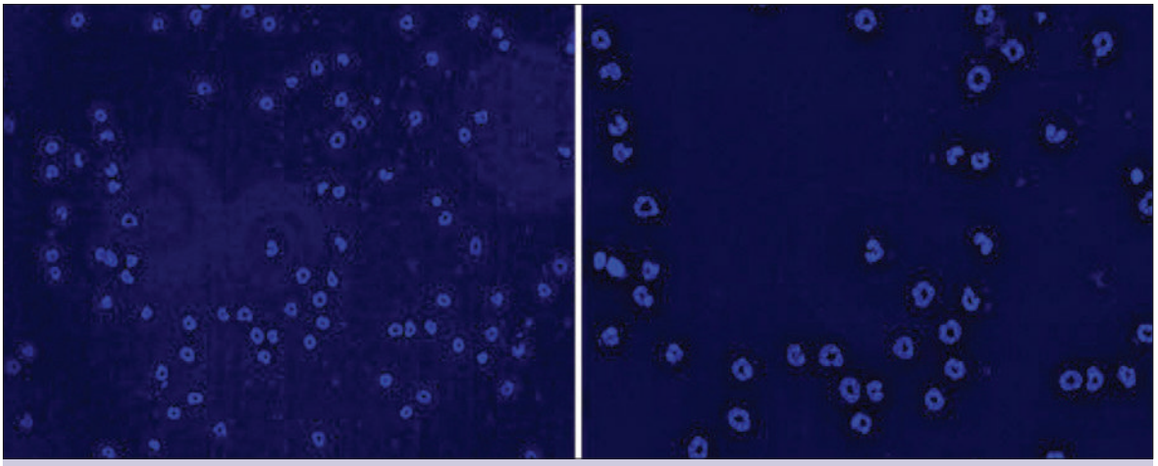
III) obtaining the N1 and N2 secretome that will be further investigated for its effect on macrophage polarization.

RESULTS:

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



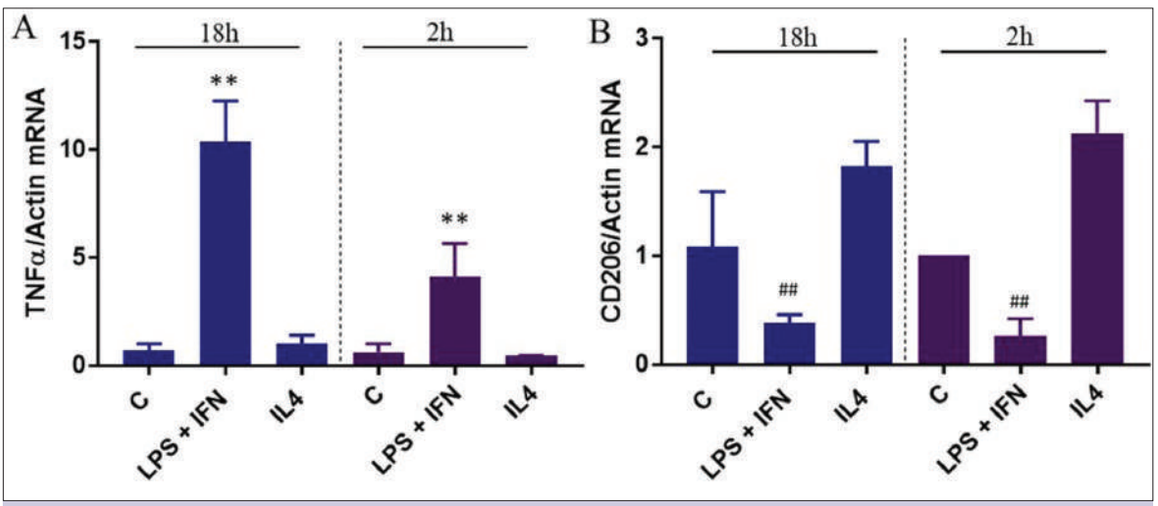
Schematic representation of the experimental procedure of the objective.



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

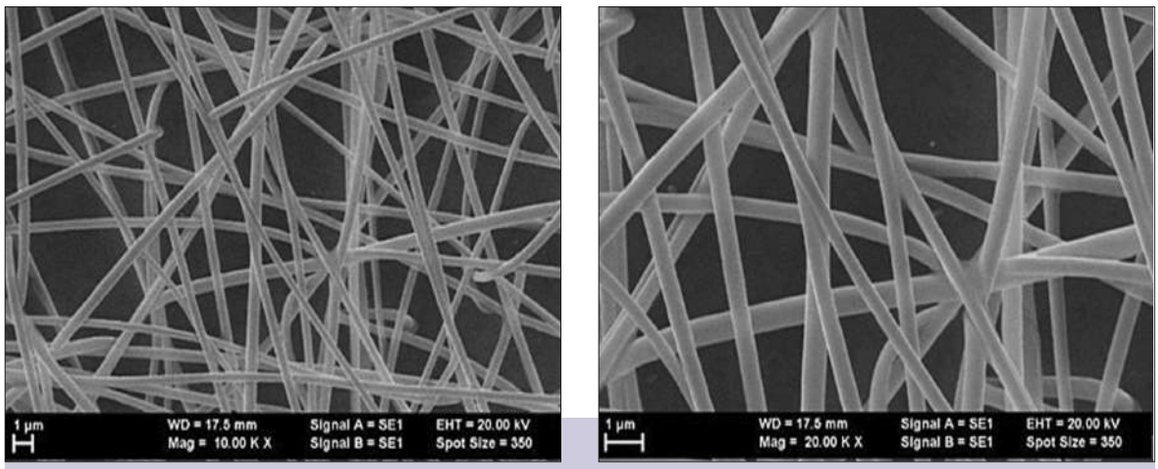
(complex project coordinator:*Maya Simionescu*), INTERA2 component project: "Development of a 3D platform designed for pre-clinical drug testing composed of cells incorporated into three-dimensional bio-matrices", project responsible: *Elena Butoi*)

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.

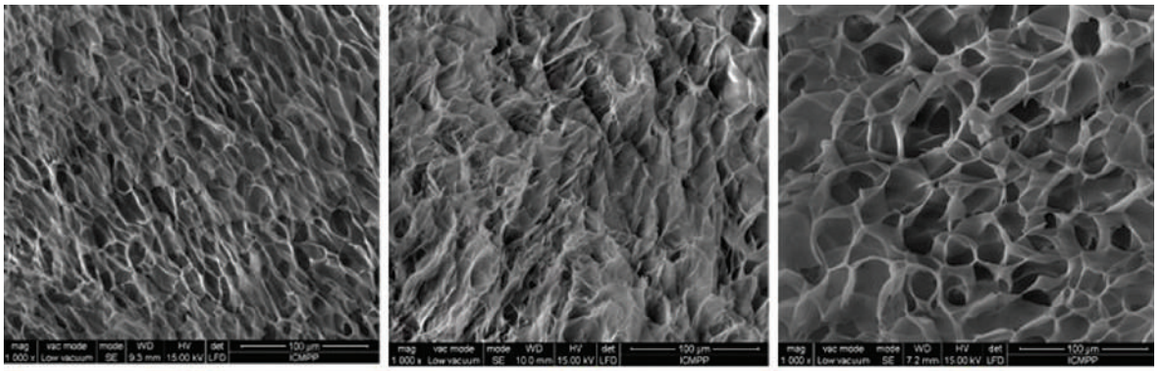
RESULTS:

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



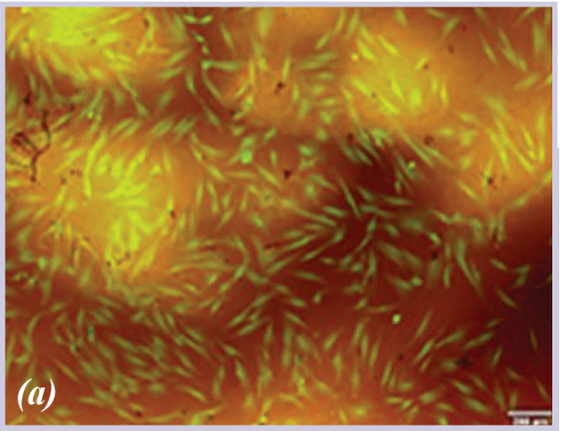
SEM images of electrospun PMMA fibers in dimethylformamide solution (porous membrane).

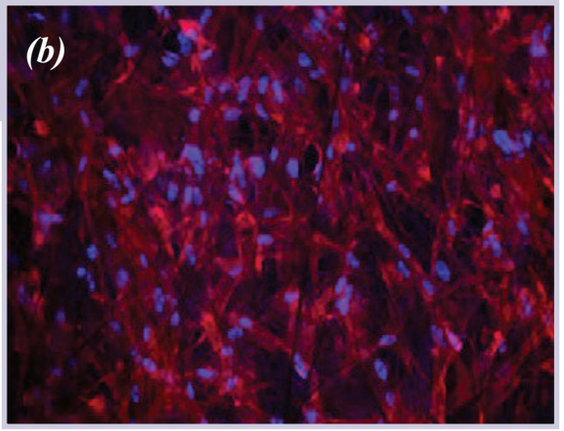
As shown, elecrospinated membranes analyzed by SEM have a fibrous morphology with fibers of about 1 micrometer.



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.*

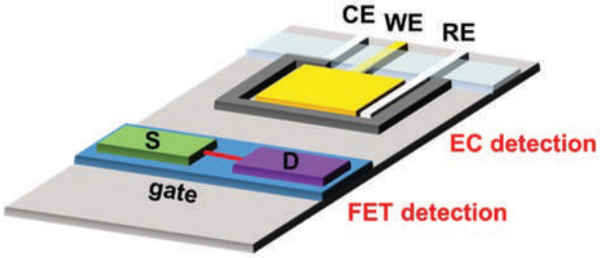
Ongoing experiments are designed to characterize the phenotype of cells cultivated on each hydrogel and to analyse the inflammatory profile on human valvular cells grown in 3D conditions compared with bi-dimensional culture.

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.

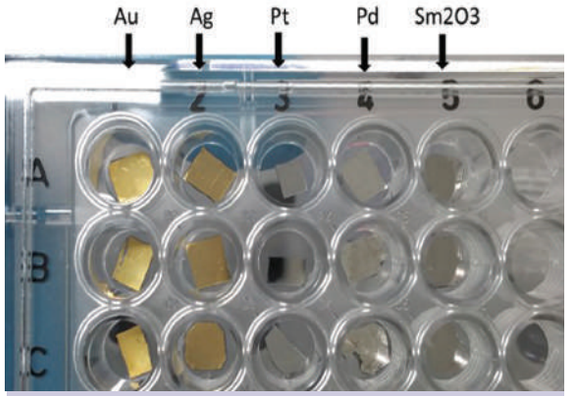


Schematic representation of the EC/ncFET detector. CE, WE, RE represents counter, working and reference electrodes. S and Dare the source and drain terminals.

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).

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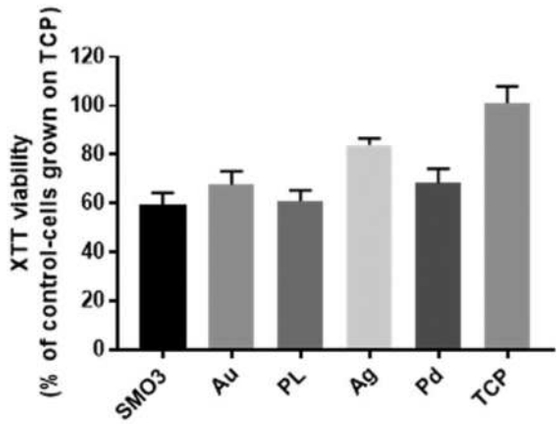

XTT assay results for endothelial cells EAhy926 grown on different metal films. Note that the silver and gold present the best viability of all investigated metals (70%, 80%, respectively from positive control, TCP).

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;