Proiect cofinantat din Fondul social European prin programul Operational Competitivitate 2014-2020
Axa prioritara 1-cercetare, dezvoltare tehnologica si inovare (CDI) in sprijinuil competitivitatii economice si dezvoltarii afacerilor
Actiunea: 1.1.4 Atragerea de personal cu competente avansate din strainatate pentru consolidarea capacitatii de CD
Titlu proiect:Terapii tintitie pentru boala valvei aortice in diabet - **Theravaldis**
MY SMIS: 104362, Contract de finantare 115/13.09.2016 (Buget: 8.657.500 lei)

Romanian Academy
Institute of Cellular Biology and Pathology
"Nicolae Simionescu"

Theravaldis Workshop:

"Diabetes in cardiovascular diseases; pathogenic mechanisms and targeted therapies"

November 27, 2020 at 15.00 P.M.

Institute of Cellular Biology and Pathology

"Nicolae Simionescu"

Organized within POC ID: 37-298

TheraValdis

Project Title: TARGETED THERAPIES FOR DIABETES-RELATED AORTIC VALVE DISEASE
Coordinator: Dr. Agneta Simionescu, Clemson University, USA
Romanian Coordinator: Dr. Ileana Manduteanu, ICBP "N. Simionescu"

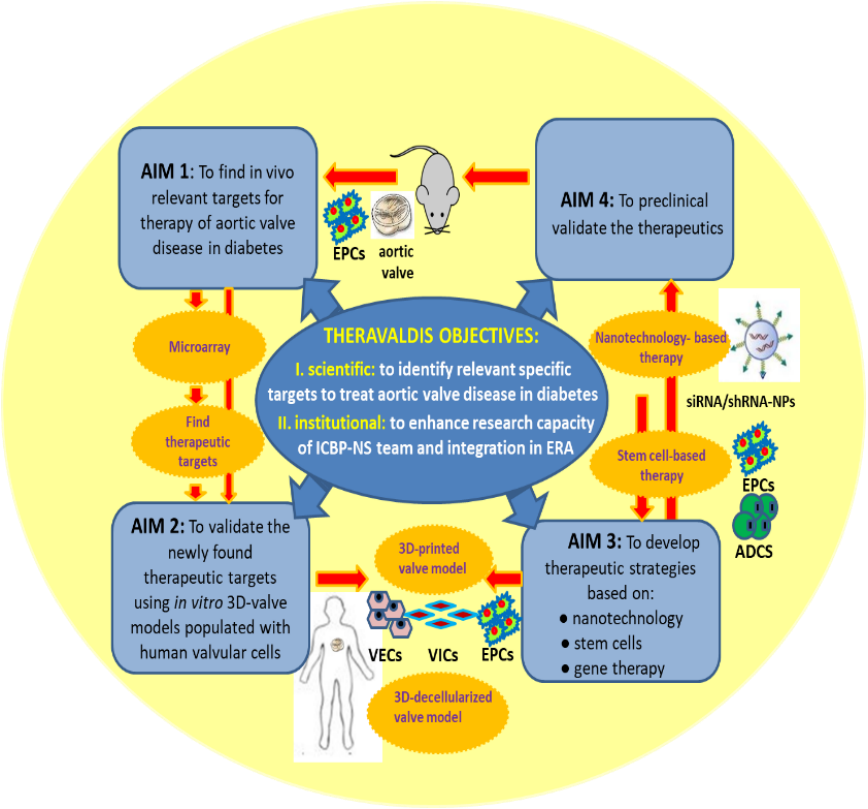
STATE OF THE ART: Aortic valve disease and especially calcific aortic valve disease (CAVD) is a global health burden in all aging societies, including the Romanian population. It is known that the presence of diabetes accelerates CAVD, and is predictive of poor prognosis in valve disease and of faster 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.

GOAL: to increase Romanian research at EU level in the field of medical and pharmaceutical biotechnology by creating a nucleus for research in nanotechnologies in ICBP "N Simionescu"

OBJECTIVE: to identify the specific mechanisms of valvular disease progression and the development of new nano-biotherapeutics for diabetes-aortic valve disease.

Schematic plan of the THERAVALDIS project objectives and aims for achieving its goal:

EXPECTED RESULTS: (i) increase of the scientific performance and excellence of ICBP-NS (ISI papers, patents) and implementation of translational medicine; **(ii)** new solutions to treat diabetes-related valve diseases contributing to improved efficiency of medical services and socially, to the health problem of the population; **(iii)** integrating ICBP in the European Area of Research by enhancing and creating new collaboration with European partners (program „Horizon 2020") and with private medical-pharmaceutics and bio-medical industry; **(iv)** better and efficient exploitation of human potential in ICBP, motivate young people by creating new jobs, reducing *„brain-drain"* and attracting researchers from abroad.



THERAVALDIS WORKSHOP PROGRAM:

"Diabetes in cardiovascular diseases; pathogenic mechanisms and targeted therapies"
27 noiembrie 2020, 15.00 - 19.00, Institute of Cellular Biology and Pathology "N. Simionescu"

SESSION I: 15.00 - 16.30 (Chairs: Agneta Simionescu, Ileana Manduteanu)

Agneta Simionescu: Introductory remarks. Mitral valve tissue engineering.
Ionel Droc: Therapeutic strategy in abdominal aortic aneurysms - is endovascular treatment the actual gold standard?
Elena Butoi: Using a 3D hydrogel derived from cell -free native aortic root as a platform for ADSC integration; therapeutic strategies for valve repair.
Manuela Călin: Preclinical evaluation of shRNA-RUNX2 nanocarriers designed to target the diseased aortic valve.

BREAK: 17.00 - 17.15

SESSION II: 17.15 – 19.00 (Chairs: Manuela Călin, Dan Simionescu)

Alexandru Filippi: Evaluation of the effects of genetically modified allogeneic endothelial progenitor cell therapy on the structure and function of the aortic valve in diabetes associated with atherosclerosis.
Dan Simionescu: Reconstitution of the ventricular endocardium and the vascular endothelium within whole acellular hearts
Ileana Mândușeanu: General Discussions and Conclusions

Poster list - THERAVALDIS Workshop:

1) **Down-regulation of RUNX2 by C60-PEI/shRNA-RUNX2 polyplexes reduces the osteodifferentiation of valvular interstitial cells.**
   Geanina Voicu, Cristina Ana Mocanu, Daniela Rebleanu, Agneta Simionescu, Maya Simionescu, Ileana Manduteanu, Manuela Calin
2) **Characterization of valvular cells in a 3D model of valvular leaflet based on a hydrogel derived from cell-free native aortic root.**
   Vadana Mihaela, Sergiu Cecoltan, Razvan Daniel Macarie, Ionel Droc, Agneta Simionescu, Dan Simionescu, Elena Butoi, Ileana Manduteanu
3) **Overexpression of RUNX2 and SMAD3 transcription factors up-regulates osteogenic, inflammation and oxidative stress -related genes in human aortic valve endothelial and interstitial cells.**
   Mihaela-Loredana Vlad, Ariana Hudita, Mihaela Vadana, Andreea Mihaila, Letitia Ciortan, Adrian Manea

PLATFORM PRESENTATIONS

Mitral Valve Tissue Engineering

Agneta Simionescu, *Clemson University, Department of Bioengineering, Clemson, SC*

Repair and replacement are current treatments for mitral valve pathologies, but they are often associated with thromboembolic complications, calcification, and the risk of additional reoperations. A tissue engineered option is feasible and holds great potential. The aim of this study was to develop an acellular, noncytotoxic mitral valve scaffold. Porcine mitral valves were treated with detergent-based solutions to remove cells, while leaving a well-preserved extracellular matrix. In order to stabilize the matrix components and slow down their degradation, scaffolds were treated with penta-galloyl glucose (PGG), a well-characterized polyphenol with high affinity for collagen and elastin. Scaffold characterization included biaxial mechanical testing of the annulus and leaflets, thermal denaturation evaluation, histological analysis, as well as resistance to collagenase and elastase. Scaffolds were seeded with human fibroblasts. PGG-treated and non-treated scaffolds were compared based on expression of TGF-β1, matrix metalloproteinases (MMPs) and α-smooth muscle actin (α-SMA). The constructs were mounted in a bioreactor for four weeks and their conditioning was analyzed using immunohistochemistry. The results show that, by maintaining the ECM structural integrity, PGG prevents the activation of cells, and consequently the weakening of tissue-engineered mitral valve, dysfunction, and disease. Cell-seeded scaffold conditioned in the bioreactor showed good viability and cell differentiation.

Therapeutic strategy in abdominal aortic aneurysms - is endovascular treatment the actual gold standard?

Ionel Droc MD PhD, *Head of Vascular Surgery Dpt. Army's Center for Cardiovascular Diseases*

Endovascular repair has emerged as an alternative to open repair for patients with abdominal aortic aneurysm. Although the method's safety and efficacy have been established, challenging anatomy and especially inadequate landing zones create limitations to its application. Stent grafts, fenestrated and branched, were developed to overpass these anatomic restrictions. Contrast-enhanced Ultrasound (CEUS) is investigated as a novel, noninvasive technique that can be employed to characterize endoleak type and consequently prescribe appropriate treatment in the follow up of these procedures.

Open repair is a safe and durable procedure. But it has some long-term complications as graft thrombosis, infection or paraanastomotic aneurysms. Aorto enteric fistula is a rare but difficult to treat complication. Also, open surgery is used for graft explantation for graft failure or infection. The overall mortality for open procedures is about 4,5%.

In conclusion, open surgery for AAA remains the gold standard in young and low risk surgical patients. Endovascular procedures are of first choice in all high-risk patients, even in emergency situations.

Using a 3D hydrogel derived from cell-free native aortic root as a platform for ADSC integration; therapeutic strategies for valve repair

Elena Butoi *Institute of Cellular Biology and Pathology "Nicolae Simionescu" of Romanian Academy*

Adipose-derived stem cells (ADSCs) are easily to obtain and expand, and have emerged as a novel source of adult stem cells for the treatment of cardiovascular diseases. ADSCs are cells capable of self-renewal and differentiation into a variety of phenotypes, and could be used for the treatment of heart failure. By releasing a series of paracrine factors, they promote neovascularization, reduce apoptosis, and inhibit fibrosis, which contributes to cardiac regeneration. As a novel therapy in the regenerative field, ADSCs still present various limitations, such as low survival and engraftment. Because of these limitations, researchers are looking for strategies to enhance cell survival and retention. In this study we have investigated the phenotype and functions of ADSC, as well as their capacity to differentiate in endothelial cells using 3D hydrogel derived from cell -free native aortic root, in order to provide therapeutic strategies for valve pathologies.

Our studies investigated ADSC survival and engraftment in a new 3D model for aortic valve leaflet (VIP), developed from native porcine tissue. Different investigations such us cell adhesion, infiltrations, cell morphology and phenotype were

performed in static or dynamic conditions using 3D model with or without valvular cell. The capacity of ADSC to differentiate towards endothelial cells was also investigated using viral transfections to overexpress integrins α4 and β1, as well us by exposure of ADSC to conditioned media from valvular endothelial cells (VECs).
The results showed that ADSCs adhered in significantly higher numbers to the VIP-hydrogel vs gelatine-based hydrogel (GP-1), and developed rapid (2 hours) a specific phenotype, in the case of VIP. Dynamic conditions lead to increased proliferation of ADSC compared to the static condition, a result that correlates with lower LDH levels in the dynamic condition. When 3D model had VEC on the surfaces, a higher number of ADSC adhered to VIP with VEC compared to GP-1 with VEC, in static or dynamic conditions. Adhesion of ADSC in continuous laminar flow conditions, in presence of SDF-1, lead to a large number of recruited ADSCs. Genetically modified ADSCs, to over-express α4 and β1 integrins, exhibited an increased adhesion compared with control ADSC. Co-cultivation of ADSCs with VIP 3D model, increased expression of ICAM-1 and of MCP-1and IL-8, suggesting the improved response capacity and metabolism of ADSC under co-cultivation with 3D -VIP constructs.
Exposure of ADSCs cultured in 2D or VIP-3D model, to conditioned medium from VECs, increased endothelial cell markers CD31 and vWF and decrease vimentin and α - SMA (considered markers of interstitial valve cells), only in 3D model.
All these data emphasize the superiority of the VIP hydrogel formula in recruiting ADSC compared to GP-1 hydrogel and underline the plasticity of ADSC when are exposed to a proper environment. The excellent survival, recruitment and ability to differentiate in 3D model of ADSC, give hope that these cells could be a promising means of treating cardiovascular disease in the near future.
This work was supported by a grant from the Competitiveness Operational Program 2014-2020, Targeted therapies for aortic valve disease in diabetes, THERAVALDIS, ID P_37_298, MySMIS code: 104362, contract number 115/13.09.2016.

The innovative nanotherapeutics designed for targeted delivery of shRNA to diseased aortic valve

Manuela Calin *Institute of Cellular Biology and Pathology "Nicolae Simionescu" of Romanian Academy*

Accumulating data suggest that diabetes induce pro-inflammatory processes that accelerate the development of calcific aortic valve disease (CAVD). An important role in CAVD is played by the osteogenic differentiation of valvular interstitial cells (VIC).
We aimed to develop suitable nanocarriers designed for targeted delivery of short hairpin (sh) RNA to the diseased aortic valve to down-regulate the expression of transcription factors validated as relevant for the progression of CAVD in diabetes. The preclinical evaluation comprises besides in vitro studies, in vivo studies performed on hyperlipidemic ApoE-deficient mice with diabetes. We demonstrated an increased expression of proteins involved in the activation and osteodifferentiation of VIC induced by exposing cells to media containing high glucose and osteogenic factors (HGOM). Next, we down-regulated the expression of transcription factor Runx2 in VIC by treating the HGOM-activated VIC with nanoparticles, namely lipopolyplexes targeted to VCAM-1 or collagen IV, carrying shRNA-Runx2 plasmids. The silencing of Runx2 determined the significant reduction of proteins involved in the activation and osteodifferentiation of VIC (e.g. bone morphogenetic protein 4, osteopontin, bone sialoprotein and alkaline phosphatase). The investigations of the therapeutic effects of VCAM-1 and collagen IV targeted nanoparticles carrying shRNA-Runx2 in a murine model of hyperlipidemic ApoE-deficient mice with diabetes showed a specific accumulation of nanoparticles in the aortic root (IVIS imaging) and downregulation of Runx2 (real-time PCR and immunofluorescence). Also, a reduced expression of mRNA for alkaline phosphatase, osteopontin and osteocalcin was found in aortic valve samples isolated from animals treated with dual-targeted nanoparticles, carriers of shRNA-Runx2.
In conclusion, dual-targeted nanocarriers directed to both VCAM-1 and collagen IV are efficient vectors for the delivery of shRNA plasmids to the affected aortic valve.

Early Diabetes Induces Alterations in Endothelial Progenitor Cell Phenotype and Homing In A Murine Model Of Atherosclerosis

Alexandru Filippi[1], Alina Constantin[1], Nicoleta Alexandru[1], Cristina Mocanu (Constantinescu)[1], Madalina Fenyo[1], Agneta Simionescu[2], Dan Simionescu[2], Ileana Manduteanu[1], Adriana Georgescu[1]
[1]Institute of Cellular Biology and Pathology 'Nicolae Simionescu' of the Romanian Academy, Bucharest, Romania, [2]Clemson University, Clemson SC, USA

The number and function of endothelial progenitor cells (EPCs) are reduced in diabetes, contributing to deteriorated vascular repair and the occurrence of cardiovascular complications. In diabetes, EPCs are sequestrated in the bone marrow by integrin α4β1 (VLA4) phosphorylation induced by hyperglycaemia and, as our previous results shown, circulatory EPCs have reduced VLA4 levels in diabetic animals.
Here we explore the treatment of early dyslipidaemic or diabetic dyslipidaemic mice with EPCs from healthy animals or EPCs from diabetic and/or dyslipidaemic mice, modified ex vivo to express GFP or overexpress VLA4. EPCs were administered on day 8 after the first streptozotocin injection used to induce diabetes or sham injection with citrate buffer. After 3 more days, echocardiography measurements were made and the animals were sacrificed the next day and the hearts and whole blood were collected. To assess the distribution of intravenously administered EPCs in target tissues, organ imaging of PKH26-stained EPCs was performed and the cytotoxicity was evaluated by ALT/AST and creatinine measurement in treated mice. Plasma lipids were evaluated for all animals. Aortic valve sections were made after heart tissue cryopreservation and used in immunohistochemical analyses of inflammatory (P-selectin, fibronectin) and remodelling markers (BMP2, αSMA, MMP9, CD31).
Our results show glycaemia levels indicative of diabetes in streptozotocin-injected animals and dyslipidaemia in all treatment groups with diabetic animals more affected. Fluorescence imaging of organs showed that EPCs are not significantly retained in the lungs after the administration, they are distributed in highly vascularised tissues and are moderately removed from the circulation in the spleen. Aortic valve velocity and velocity time integral were significantly increased and the valve opening was decreased, suggestive of aortic valve stenosis, in animals injected with EPCs from diabetic and/or dyslipidaemic mice compared with those injected with healthy EPCs, and VLA4 overexpression on EPCs recovered these parameters to the values seen in the controls. Microscopy analysis showed how the inflammatory and remodelling markers were associated with the aortic valve function.
In conclusion, our experiments show that genetically modified allogeneic EPCs could be a safe treatment option, with bioavailability in the desired target compartments and the ability to improve aortic valve function of dyslipidaemic or diabetic dyslipidaemic animals.

This work was supported by a grant from the Competitiveness Operational Program 2014-2020, Targeted therapies for aortic valve disease in diabetes, THERAVALDIS, ID P_37_298, MySMIS code: 104362, contract number 115/13.09.2016.

Reconstitution of the ventricular endocardium and the vascular endothelium within whole acellular hearts

Dan Simionescu, Clayton Compton, Martin Groke, Jessica Canavan, John Mcleod, Connor Prevost, Jason Schulte, Megan Casco, Margarita Portilla, Benjamin Fisher, Agneta Simionescu
Department of Bioengineering, Clemson University, Clemson, SC, USA

OBJECTIVES: There is a significant clinical need for developing living, tissue engineered whole hearts for transplantation. To address this need we pursued the creation of biological scaffolds through decellularization of whole hearts and seeding them with the appropriate autologous cells for full reconstruction. Bulk seeding of whole organs such as the heart is an enormous challenge because of its great three-dimensional anatomical and biological complexity. To overcome these challenges, we are developing techniques that target the specific reconstitution of major anatomical components of the heart. These techniques encompass combinations of perfusion bioreactors, flow loops, hydrogels, adhesive proteins and other tissue engineering methodologies.
AIMS: Once an acellular heart is connected to the host circulation, the first concern would be thrombogenicity of the blood contacting surfaces; therefore, regeneration and re-endothelialization of the endoluminal (intimal) surfaces appear to be the first logical targets for cell seeding. The specific aim of the current study was to reconstitute and reendothelialize the endocardial layer of the left and right ventricular cavities, the surfaces of the interventricular septum and the coronary vasculature of whole acellular hearts.
METHODS: Whole rabbit hearts were cannulated, mounted on a manifold and fully decellularized using a biventricular perfusion system. The extent of decellularization was assessed by evaluating presence of cells (DAPI stain, DNA content) and preservation of matrix composition (Trichrome, VVG stains and immunohistochemistry). For endocardial reconstitution, we designed, and 3D printed a support system for the rabbit cardiac scaffolds and seeded the two ventricular cavities with human fibroblasts in collagen hydrogels followed by human endothelial cells suspended in fibrin hydrogels, in a layer-by-layer fashion. After seeding we conditioned the constructs in a rotational bioreactor for 3 days. Tissues were analyzed by histology, immunohistochemistry and scanning electron microscopy. In a separate study, the coronary vasculature was perfused with living endothelial cells for 24 hours using a closed flow loop system followed by recirculation of media for another 48 hours to remove loosely bound cells and tissues analyzed by histology.

RESULTS: Complete decellularization of numerous whole rabbit hearts was successful using our manifold system. Hydrogels infused well onto most ventricular trabecular surfaces and pores of the scaffold. Seeded cells adhered to many areas of the two ventricles while remaining active by secreting new matrix proteins such as laminin. The acellular hearts had a fully perfusable vasculature which allowed seeding of endothelial cells via the coronary arteries. Reendothelialization of the coronary vasculature was effective but incomplete.
CONCLUSIONS: These results indicate that regeneration of whole organs such as the heart can be achieved using a site-specific seeding approach that takes functional tissue architecture into consideration. Current research is focused on spatial seeding of cardiomyocytes within acellular hearts using novel seeding devices and lastly combining all these approaches to fully regenerate whole hearts before implantation.
ACKNOWLEDGMENTS: Funding was provided by NIH grants 1P30GM131959, 1R56HL130950, 1R01HL133303 and by the Harriet and Jerry Dempsey Bioengineering Professorship Award.

POSTER PRESENTATIONS

1. DOWN-REGULATION OF RUNX2 BY C60-PEI/shRNA-RUNX2 POLYPLEXES REDUCES THE OSTEODIFFERENTIATION OF VALVULAR INTERSTITIAL CELLS

Geanina Voicu[1], Cristina Ana Mocanu[1], Daniela Rebleanu[1], Agneta Simionescu[2], Maya Simionescu[1], Ileana Manduteanu[1], Manuela Calin[1]
[1]*Institute of Cellular Biology and Pathology "N. Simionescu", Bucharest, Romania;*
[2]*Department of Bioengineering, Clemson University, United States of America*

Background. Calcific aortic valve disease (CAVD) is a progressive disorder that increases in prevalence with age. An important role in aortic valve calcification is played by valvular interstitial cells (VIC), that with age or in pathological conditions acquire an osteoblast-like phenotype that advances the disease. Therefore, pharmacological interventions aiming to stop or reverse the osteoblastic transition of VIC may represent a therapeutic option for CAVD.
Aim. To develop a nanotherapeutic strategy able to prevent the phenotypic switch of human aortic VIC into osteoblast-like cells. We hypothesize that nanocarriers designed for silencing the Runt-related transcription factor 2 (Runx2) will stop the progress or reverse the osteodifferentiation of human VIC, induced by high glucose concentrations and pro-osteogenic factors.
Methods. The polyplexes were characterized by size, zeta-potential, complexation efficacy, cytotoxicity and uptake in VIC. Cultured VIC were exposed to media containing normal (NG, 5.5 mM) or high (25 mM) glucose concentrations in the presence of factors (HGOM) known to induce osteodifferentiation (10 nM dexamethasone, 10 mM β-glycerophosphate, 50 µg/ml L-ascorbic acid). After 5 days, VIC were transfected with C60-PEI/shRNA-Runx2 polyplexes containing plasmids with shRNA sequences specific for Runx2 (N/P ratio of 25) and the levels of Runx2, BMP4, ALP, BSP, OSP were determined by RT-PCR or Western Blotting assay at 48 hours after transfection. The ALP activity was enzymatically analyzed at 7, 14 and 21 days in VIC exposed to HGOM and transfected with polyplexes. For 14 and 21 days, the cells were transfected twice with the same type of polyplexes on the 5th and 12th days.
Results. We report here the potential of fullerene (C60)-polyethyleneimine (PEI)/short hairpin (sh)RNA-Runx2 nano-polyplexes to efficiently down-regulate Runx2 mRNA and protein expression leading subsequently to a significant reduction in the expression of osteogenic proteins (i.e. ALP, BSP, OSP and BMP4) in osteoblast-committed VIC.
Conclusions. The data suggest that the silencing of Runx2 could represent a novel strategy to impede the osteoblastic phenotypic shift of VIC and the ensuing progress of CAVD.
This work was supported by a grant from the Competitiveness Operational Program 2014-2020, Targeted therapies for aortic valve disease in diabetes, THERAVALDIS, ID P_37_298, MySMIS code: 104362, contract number 115/13.09.2016.

2. CHARACTERIZATION OF VALVULAR CELLS IN A 3D MODEL OF VALVULAR LEAFLET BASED ON A HYDROGEL DERIVED FROM CELL-FREE NATIVE AORTIC ROOT

Vadana Mihaela[1], Sergiu Cecoltan[1], Razvan Daniel Macarie[1], Ionel Droc[2], Agneta Simionescu[3], Dan Simionescu[3], Elena Butoi[1], Ileana Manduteanu[1]
[1]*Institute of Cellular Biology and Pathology "N. Simionescu", Bucharest, Romania;*
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Background. Heart valve disease is an increasingly prevalent and clinically serious condition and has no pharmacotherapy. The only remedy available is replacement with a prosthetic valve, but the inability of these devices to grow or respond biologically to their environments necessitates multiple resizing surgeries and life-long coagulation treatment.
Aim. Tissue engineering has a unique opportunity to impact heart valve disease by providing a living valve conduit, capable of growth and biological integration. In this context we have developed a valvular hydrogel from porcine valve tissue and populated it with human valvular cells: interstitial valvular cells (VIC) were encapsulated within the hydrogel and endothelial valvular cells (VEC) were seeded on the top.
Methods. Cell were allowed to grown in the 3D model, next characterized by cell viability, histological staining, fluorescence (for cell morphology) and by immunofluorescence on the constructs or on sections (for expression of different specific markers for valvular cells).
Results. The morphology and bio-compatibility screening showed that the valve cells in the 3D model have a specific morphology and are viable over time. Moreover, the viability of endothelial cells on the surface of the obtained model is 4 times higher at 11 days compared to 3 days after cultivation. The results showed that started with second day, encapsulated VIC exhibited a fibroblast-like phenotype, became elongated and interconnected, forming complex cell networks. VEC seeded on top proliferate and form a monolayer over the hydrogel. Both valvular cells from 3D valve model exhibits specific markers, with CD31 expressed by VEC, and vimentin and α-SMA expressed by VIC. Moreover, VIC are metabolic active and exhibits a contractile phenotype, contracting the construct over 50% in 48h.
Conclusions. These data highlight that the developed model has excellent biocompatibility with interstitial and endothelial valve cells, making it ideal as a model to study the cellular and molecular mechanisms of aortic valve pathologies.
This work was supported by a grant from the Competitiveness Operational Program 2014-2020, Targeted therapies for aortic valve disease in diabetes, THERAVALDIS, ID P_37_298, MySMIS code: 104362, contract number 115/13.09.2016.

3. Overexpression of RUNX2 and SMAD3 transcription factors up-regulates osteogenic, inflammation and oxidative stress -related genes in human aortic valve endothelial and interstitial cells

Mihaela-Loredana Vlad, Ariana Hudita, Mihaela Vadana, Andreea Mihaila, Letitia Ciortan, Adrian Manea
Institute of Cellular Biology and Pathology "N. Simionescu", Bucharest, Romania

Background. The expression levels RUNX2 and SMAD3 transcription factors are increased in the aortic valves of diabetic patients. Mechanistically, RUNX2 is involved in the process of osteoclast differentiation while the activation of SMAD3 induces pro-fibrotic effects. Yet, the precise down-stream molecular effectors of RUNX2 and SMAD3 underlying aortic valve stenosis in diabetes are not entirely elucidated.
Aim. In this context, the aim of this study was to identify potential inflammation and oxidative stress-related molecular targets of these transcription factors.
Methods. Cultured human aortic valve endothelial cells (VEC) and interstitial cells (VIC) were used. The cells were transiently transfected with pCMV6-RUNX2, pCMV6-SMAD3 or pCMV6-Entry (empty vector, negative control) using polyethyleneimine (ViromerTM RED) polyplex technology. Real-time PCR analysis was employed to determine the gene expression levels of selected osteogenic (osteocalcin), pro-inflammatory (TNFα, MCP-1) and oxidative stress-related genes (Nox1, Nox2, Nox4, Nox5).
Results. Gene expression analysis confirm the robust up-regulation of RUNX2 and SMAD3 mRNA levels in both VEC and VIC following transfection of the cells with pCMV6-RUNX2 or pCMV6-SMAD3 expression vector, respectively. Significant increases in osteocalcin transcript level were detected in both VEC and VIC overexpressing RUNX2 or SMAD3. Up-regulation of RUNX2 significantly induced the mRNA of inflammation and oxidative stress-related genes in cultured VEC (TNFα, Nox4) and VIC (TNFα, MCP-1, Nox1, Nox5).
Conclusions. The data of this study provide evidence that activation of RUNX2 and SMAD3 transcription factors mediates the expression of key genes mechanistically linked to inflammation and oxidative stress that may induce phenotypic alterations of VEC and VIC and potentially accelerate the process of aortic valve calcification in diabetes.
This work was supported by a grant from the Competitiveness Operational Program 2014-2020, Targeted therapies for aortic valve disease in diabetes, THERAVALDIS, ID P_37_298, MySMIS code: 104362, contract number 115/13.09.2016.

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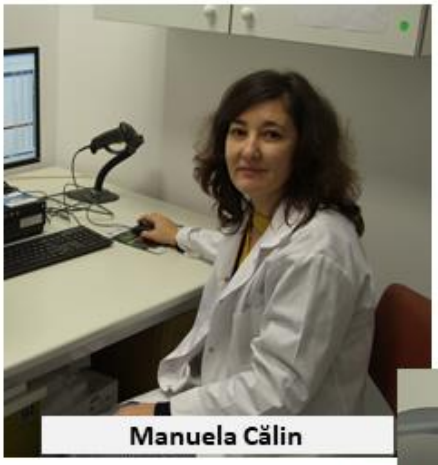

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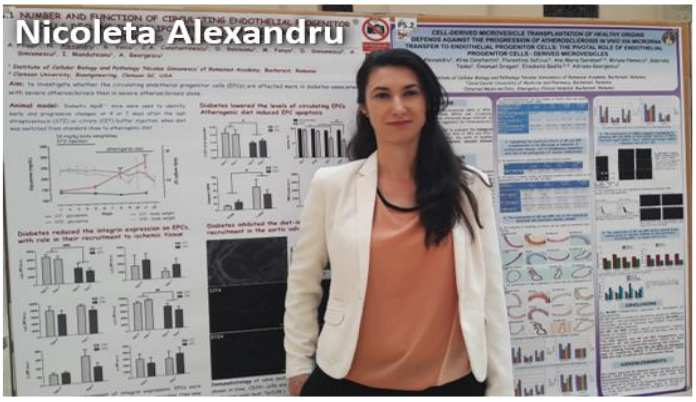
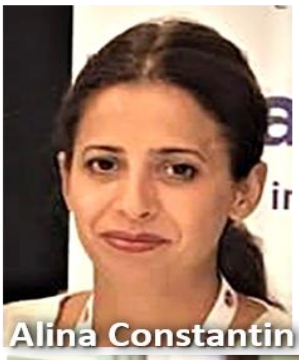
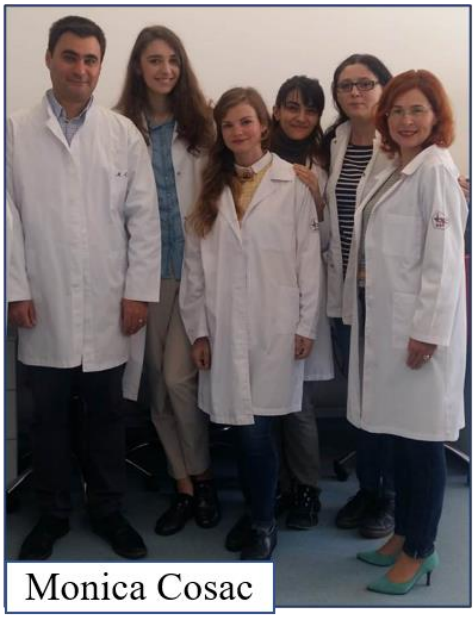




Molecular and Cellular Pharmacology - Functional Genomics Laboratory


Loredana Antonescu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