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Proceedings | *Resumos*

LIAC MEETING ON VASCULAR RESEARCH

9 a 12 September | 9 a 12 Setembro
Lisbon U. Lusofona's | Lisboa - Universidade Lusófona

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Program /Programa

9 September | *9 de Setembro*

Open Session | *Sessão de abertura*

António Tamburro Conference
Lisboa's Wine & Cheese battle

10 September | *10 de Setembro*

1st Session |*Sessão 1*
Molecular and Supramolecular Structure | *Estrutura Molecular e Supramolecular*

Charmain | *Moderador* **- Alain Pierre Gadeau**
Keynote lecture 1 | *1ª Conferência Keynote*
Speaker | *Prelector*
Sylvie Ricard-Blum

Free Comunications | *Comunicações livres*
Speakers | *Prelectores*
Brigida Bochicchio
Vicenta Llorente-Cortés
Zeinab El Dirani
J Leal Monedero

Keynote lecture 2 | *2ª Conferência Keynote*
Speaker | *Prelector*
Valerie Samouillan

Program /Programa (cont.)

2st Session |*Sessão 2*
Cell Biology and Signaling | *Biologia Celular e Sinalização*
Charmain | *Moderador* **- Michel Spina**
Keynote lecture 3 | *3ª Conferência Keynote*
Speaker | *Prelector*
Vicente Andrés

Free Comunications | *Comunicações livres*
Speakers | *Prelectores*
Alain Gadeau
Cristina Sena
Filipe Paula
Pascal Maurice

11 September | *11 de Setembro*

3st Session |*Sessão 3*
Biomaterials and Tissue Engineering | *Biomateriais e Engenharia de Tecidos*
Charmain | *Moderador* **- Brigida Bochicchio**
Keynote lecture 4 | *4ª Conferência Keynote*
Speaker | *Prelector*
Livia Visai

Free Comunications | *Comunicações livres*
Speakers | *Prelectores*
Gabriele Corsaro
Michel Spina
Ricardo Moreira

Keynote lecture 5 | *5ª Conferência Keynote*
Speaker | *Prelector*
Antonio D'Amore

4st Session |*Sessão 4*
Innovation and Technology from Diagnostics to Therapeutics | *Inovação e Tecnologia de Diagnóstico à Terapêutica*
Charmain | *Moderador* **- Philippe Charpiot**
Keynote lecture 6 | *6ª Conferência Keynote*
Speaker | *Prelector*
Laurent Riou

Free Comunications | *Comunicações livres*
Speakers | *Prelectores*
Carlota Saldanha
Eduardo Vilela
Hugo Ferreira

Keynote lecture 7 | *7ª Conferência Keynote*
Speaker | *Prelecto*
Geoffrey Mitchell

12 September | *12 de Setembro*

5st Session |*Sessão 4*
Clinical Applications | *Aplicações Clínicas*
Charmain | *Moderador* **- Vicenta Llorente-Cortés**
Free Comunications | *Comunicações livres*
Speakers | *Prelectores*
Antonio Leppeda
Henrique Silva
Diogo Fonseca

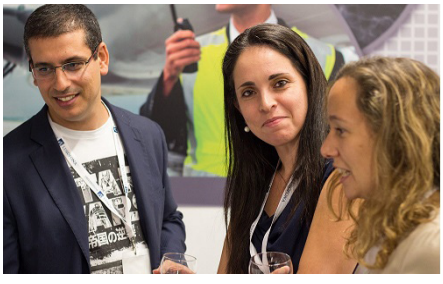
Open Session

From Left to Right / Da esquerda para a direita:
Alexandre Delgado
Michel Spina
Luís Monteiro Rodrigues

ANTONIO TAMBURRO Conference

"The Symphony in Portugal" – Alexandre Delgado, The Center for Information and Research on Portuguese Music, Lisboa Portugal
Alexandre Delgado was born in Lisboa, in 1965 and develops his work in music as an interpreter (violin) and as a composer. His extraordinary career has many landmarks. It includes a varied formation as a student, at the National Conservatory of Music, several scholarships and masterclasses, and many distinctions, namely the prize "Young Musicians" (1987) and the prize "João de Freitas Branco" (1992). His work as a composer includes several instrumental and orchestral pieces and, more recently, two operas. Alexandre Delgado actually develops an intense social activity within his expertise area, as a cultural promoter (national radio, television) but also as a critic as well, being thoroughly recognized as one of the most notorious and prestigious studious of Portuguese chamber and orchestral music.

Lisboa's Wine & Cheese battle



1st Session | Sessão 1
Molecular and Supramolecular Structure | *Estrutura Molecular e Supramolecular*

Chairman / *Moderador*

Alain Pierre Gadeau

Key-note lecture 1 - The collagens: tissue designers and regulator of biological processes (U Lyon)

Sylvie Ricard-Blum

Resumé / *Currículo Resumido*

Professor of Biochemistry at the University of Lyon and leader of the team "Extracellular interaction networks". Her group has created a pipeline to build ECM networks regulating angiogenesis, Alzheimer's disease and host-pathogen interactions, and a database, MatrixDB, to store ECM interaction data. She has been President of the French Society for Matrix Biology for 5 years, is a member of the council of the International Society for Matrix Biology and of the Editorial board of Matrix Biology.

Abstract / *Resumo da Comunicação*

UMR 5086 CNRS - University Lyon 1, Lyon, France

Twenty-eight collagen types have been identified in mammals. They all contain a triple-helical domain, which is a characteristic feature of the collagen superfamily. Several collagen types (e.g. collagens I, II, III, V) form fibrils, whereas others do not form fibrils by themselves but associate with fibrils (e.g. collagen IX) or form networks (collagen IV), beaded filaments (collagen VI) and anchoring fibrils (collagen VII). Some collagens participate in different supramolecular assemblies depending on the tissue (e.g. collagen XVI) and on their interactions with other extracellular matrix (ECM) biomolecules (e.g. collagen VI). Collagen fibrils are covalently cross-linked via the lysyl oxidase(s) and by glycation, which may damage collagens and the ECM in ageing and diabetes. Cross-linking modulates the stiffness and mechanical properties (e.g. resistance to traction) of tissues but tensile overload causes discrete plasticity in collagen fibrils and the formation of kinks, which contain denatured collagen molecules.
Besides their contribution to the architecture of tissues collagens fulfill biological roles either as full-length proteins or as bioactive fragments called matricryptins. These functions are mediated by several receptor families (integrins, discoidin domain receptors, leukocyte-associated immunoglobulin-like receptors, osteoclast-associated receptor, and GPR56, an orphan G protein-coupled receptor) and associated-molecules named COLINBRIs (COLlagen INtegrin BRIdging). Collagens regulate cell growth, differentiation and migration and physiopathological processes such as atherosclerosis, fibrosis, diabetes, angiogenesis and cancer. They also play a role in the development of the central nervous system and in Alzheimer's disease.
Ricard-Blum S. The collagen family. Cold Spring Harb (2011) Perspect Biol 3:a004978
Ricard-Blum S, Salza R. Matricryptins and matrikines: biologically active fragments of the extracell

C.01 - Molecular and Supramolecular Structure of glycopeptides as scaffolds in tissue engineering (U Basilicata)

Speaker / *Prelector*

Brigida Bochicchio

Abstract / *Resumo da Comunicação*

Motivation statement: Glycopeptides are an emerging class of bioinspired polymers that mimic nature. Elastin is naturally present in extracellular matrix and it is responsible for elasticity of organs and tissues. therefore, elastin-derived peptides conjugated with carbohydrates and iminosugars are expected to be biocompatible elastomers with great potential in biomedical applications.
Methods: Glycopeptides were synthetized by conjugating a self-assembling elastin-derived peptide with carbohydrates and iminosugars via thiol-ene and oxime chemistry, respectively. Results: A molecular and supramolecular study of the synthetized biopolymers was carried out. Outsdanding differences were shown at supramolecular level thus suggesting a specific self-assembling mechanis for glycopeptides.
Conclusions: The glycopeptide was able to self-assemble thus suggesting their potential use as biomaterials.
Acknowledgments: The financial support from MIUR (PRIN 2010LSH3K) is gratefully acknowledged.

C.02 - Domain CR9 of Low-Density Lipoprotein Receptor-Related Protein 1 (LRP1) Is Critical for Aggregated LDL-Induced Foam Cell Formation from Human Vascular Smooth Muscle Cells (CSIC-ICCC, Barcelona)

Speaker / *Prelector*

Vicenta Llorente-Cortés

Abstract / *Resumo da Comunicação*

LRP1 mediates the internalization of aggregated LDL (AgLDL), which in turn increases the expression of LRP1 in human vascular smooth muscle cells (hVSMC). This positive feedback mechanism is thus highly efficient to promote the formation of hVSMC-foam cells. Here we have determined LRP1 domains involved in AgLDL recognition with the aim of specifically blocking AgLDL internalization in hVSMC. The capacity of fluorescently labeled AgLDL to bind to functional LRP1 clusters was tested in a receptor-ligand fluorometric assay made by immobilizing soluble LRP1 "minireceptors" (sLRP1-II, sLRP1-III and sLRP1-IV) recombinantly expressed in CHO cells. This assay showed that AgLDL binds to cluster II. We predicted three well exposed and potentially immunogenic peptides in CR7-CR9 domains of this cluster [termed P1, Cys1051-Glu1066, P2 (Asp1090-Cys1104) and P3 (Gly1127-Cys1140)]. AgLDL, but not native LDL, bound specifically and tightly to P3-coated wells. Rabbit polyclonal antibodies raised against P3 prevented AgLDL uptake by hVSMC and were almost twice more effective than anti-P1 and anti-P2 Abs in reducing intracellular cholesteryl ester accumulation. Moreover, anti-P3 Abs highly efficiently prevented AgLDL-induced LRP1 upregulation and counteracted the downregulatory effect of AgLDL on hVSMC migration. In conclusion, domain CR9 appears to be critical for LRP1-mediated AgLDL binding and internalization in hVSMC. Our results open new avenues for an innovative anti-VSMC-foam cell-based strategy for the treatment of vascular lipid deposition in atherosclerosis.

1st Session | Sessão 1
Molecular and Supramolecular Structure | *Estrutura Molecular e Supramolecular*

C.03 - Beneficial effects of physical training on the vascular dysfunction induced by intermittent hypoxia (U Grenoble - U Libanaise)

Speaker / *Prelector*

Zeinab El Dirani

Abstract / *Resumo da Comunicação*

Obstructive sleep apnea syndrome (OSA) is characterized by multiple breathing interruptions during sleep due to transient closing of the superior airways. This induces chronic intermittent hypoxia (IH), which enhances cardiovascular dysfunction and remodeling, potentially leading to hypertension and cardiac infarct. Since physical training was described as limiting the extent and occurrence of several cardiovascular diseases, we studied its effect on the vascular reactivity and calcium signaling in vascular smooth muscle cells (SMC) of rats exposed to IH, with the hypothesis that exercise may correct the negative impact of IH.

Wistar rats which were randomly assigned to 4 groups: N (Normoxic sedentary rats), NIT (Normoxic Intensive Trained), IH (Intermittente Hypoxia Sedentary rats), IHIT (Intermittent Hypoxia Intensive Trained rats). IH consisted in alterning normoxia (21% O2) and hypoxia (5% O2) every 30 s in the cages for 8h/day. Exercise sessions were conducted 5 times/week in NIT and IHIT rats during 3 weeks, as a fast 30 min walk with a speed progressively rising from 16 to 30 m/min. After 21 days of IH (with/without training), the dose-effects of the reference vasoactive agonists have been investigated regarding vascular reactivity, by using the tension arteriography technique in aorta rings. Also, calcium imaging of cultured aortic SMC was performed to examine the effects of IH and training on intracellular calcium signaling.

21 days of exposure to IH had no impact on aortic sensitivity to vasoconstrictors (Endothelin-1, Phenylephrine). However, IH reduced the relaxant effect of acetylcholine in the aorta and increased the caffeine-induced elevation of cytoplasmic calcium level in aortic SMC. These alterations were reduced by exercise. Our results suggest that intensive exercise training could decrease the adverse effects of IH on the cardiovascular physiology. This could lead to improvements in the treatment of OSA patients.

C.04 - Protocols for studying Pelvic Venous Pathology (Hospital Rúber Internacional, Madrid)

Speaker / *Prelector*

Leal Monedero

Abstract / *Resumo da Comunicação*

Pelvic Congestion Syndrome is a condition causes by an increase, both in number and size, of intra-pelvic venous structures. These structures typically present a varicose morphology, consisting on tortuous, dilated and ecstatic veins, with flow alterations.

The main symptom is chronic pelvic pain, with more than 6 months of evolution and non-other related cause. This pain will increment with the patient on standing position. Other symptoms of this syndrome include perineal heaviness sensation, dyspareunia, dysmenorrhea, and apparition of genital and lower limb varicose veins.

This syndrome is closely related to multiple pregnancies in women, but also to the presence of congenital compressions. The venous hypertension is considered as the main etiology, developing refluxes in gonadal veins and tributaries to internal iliac veins. Depending on the patient, and typically in cases of compromised pelvic floor, leaking points to the lower limb will appear or not.

The diagnosis is stablished using Color Duplex Ultrasounds, both Transvaginal DU and External transparietal DU, with a reliability of 96%. Other medical imaging techniques, such as CT angiogram and/or MR angiography, could be required in some cases, in order to clarify the diagnosis.

A diagnostic confirmation is undertaken using Pelvic Phlebography, allowing performing the embolization treatment of the refluxive veins in the same procedure. In some cases, a Stenting procedure is also required to treat Compressive Syndromes, as a part of the treatment of the refluxes.

It will be also necessary to study pelvic venous pathology in cases of atypical lower limb varicose veins, as previously stablished. In those cases, it will be also mandatory to treat the refluxive veins as part of treatment. Resuming, embolization therapy to treat pelvic insufficient axis is an efficient procedure, with a success rate of more than 97%, both in women and men, with minimal complications founded.

1st Session | Sessão 1

Molecular and Supramolecular Structure | *Estrutura Molecular e Supramolecular*

Key-note lecture 2 - Scanning of the supramolecular organization of the proteins of the extracellular matrix by biophysical techniques (U Toulouse)

Valerie Samouillan

Resumé / *Currículo Resumido*

Valerie Samouillan received her Ph.D degree from the University of Toulouse, France (Université Paul Sabatier) in 1999. She is assistant professor at the University of Toulouse and she is member of the CIRIMAT Institute (Inter-University Material Research and Engineering Centre) in the Polymer Physics team. Her research interests are oriented toward the structure-function relationships in biomacromolecules and biological tissues trough vibrational, thermal and dielectric analyses.

Abstract / *Resumo da Comunicação*

The main constitutive macromololecules of the extracellular matrix, essential to the cohesion and resiliency, are also active components which evolve with the physiology and pathology.
Their functionality is connected to their internal dynamics over various scales of time and lenght, in close correlation with water. The different levels of structure in freeze-dried and hydrated proteins have been explored for several decades by static techniques, giving structure/function relationship. Nevertheless, time and frequency analyses, which have showed their ability to determine the dynamics of synthetic polymers, deserve to be adapted to the study of polypeptides, proteins and tissues.
Through various examples, we will show how the dielectric technics, coupled with thermal and vibrational analyses, allow to characterize biologic systems of increasing complexity, both at the nanometric and mesocospic levels.
In the physiological state, the vibrational FTIR spectra of cardiovascular tissues or cell supernatants can be correlated to the vibrational answer of the main proteins - namely elastin and collagens- allowing to discriminate their different secondary structures. Using Differential Scanning Calorimetry (DSC), the thermal fingerprints of elastin and collagens can be detected in cardiovascular tissues, evidencing a strong correlation between chain architecture and mesophases organization. The dielectric techniques - Dynamic Dielectric Spectroscopy (DDS) and ThermallyStimulated Currents (TSC) - reveal the localized and delocalized levels of mobility of these proteins in tissues, allowing to differentiate them via their hydrogen bonds network.
This set of data collected on safe tissues and proteins can be then used to follow the evolution of thermal, vibrational and thermal signatures of proteins in pathological states and also to optimize the conception of substitutive biomaterials in the cardiovascular research.

2st Session | Sessão 2
Cell Biology and Signaling | *Biologia Celular e Sinalização*

Chairman / *Moderador*

Michel Spina

Key-note lecture 3 - Role of A-type lamins in aging and cardiovascular disease

Vicente Andrés

Resumé / *Currículo Resumido*

Dr. Andrés has made seminal contributions into the mechanisms of atherosclerosis and restenosis and the identification of biomarkers of these diseases. His research has also contributed to understanding the role of A-type lamins and telomere dysfunction in the control of gene expression, cell proliferation and signaling in cardiovascular disease (CVD) and aging. He has been awarded the Dr. Leon Dumont Prize 2010 by the Belgian Society of Cardiology.

Abstract / *Resumo da Comunicação*

The world population is experiencing progressive aging, the main cardiovascular disease (CVD) risk factor. Nuclear lamins A and C (A-type lamins, LMNA gene) are filamentous proteins with architectural and functional roles. Over 460 LMNA mutations have been linked to human diseases called laminopathies, including Hutchinson-Gilford progeria syndrome (HGPS), a rare disorder caused by a lamin A variant called progerin. HGPS is characterized by excessive atherosclerosis, vascular calcification and early death, predominantly from myocardial infarction or stroke (average lifespan: 13 yr). Progerin is also expressed in aged tissues of non-HGPS subjects, suggesting a role in normal aging. Our laboratory investigates the role of A-type lamins and progerin in aging and cardiovascular disease. To this end, we use mouse and human cells and genetically-engineered mouse models in which expression of lamin A/C and progerin is manipulated either globally or in a tissue-specific manner to uncover new and possibly cell-type-specific mechanisms governing normal and premature aging, and related atherosclerosis, vascular calcification and heart disease. We also seek to identify gender- and age-related changes in protein abundance and oxidation in organs affected in progeria and normal aging or only in normal aging with the ultimate goal of identifying molecular mechanisms common to both processes as well as specific to each.

C.05 - Down regulation of hedgehog signaling in nerves and myocytes contributes to the impaired ischemic muscle repair in elderly

Speaker / *Prelector*

Alain Pierre Gadeau

Abstract / *Resumo da Comunicação*

Background: In elderly and diabetic patients, the increased risk of developing ischemic disease is associated with impaired regenerative properties of most tissues including skeletal muscle, which make those patients a challenging population to treat. We recently demonstrated that in the skeletal muscle, Hedgehog (Hh) signaling promotes ischemia-induced angiogenesis by maintaining peripheral nerve and by promoting myogenesis. The objective of this study is to investigate the functionality of Hh dependent regulation of peripheral nerve survival and myogenesis in aged mice.
Methods and results: In the present study, we used 12 week old (young mice) and 20 to 24 month old C57BL/6 mice (old mice) to investigate the activity of Hh signaling in the setting of ischemic skeletal muscle regeneration. In this model, delayed ischemic muscle repair observed in old mice was associated with an impaired upregulation of Gli1. Sonic Hedgehog expression was not different in old mice compared to young mice while Desert Hedgehog (Dhh) expression was downregulated in the skeletal muscle of old mice both in healthy and ischemic conditions. The rescue of Dhh expression by gene therapy in old mice increased nerve density and promoted ischemia-induced angiogenesis, nevertheless it failed to promote myogenesis. After further investigation, we found that, in addition to Dhh knockdown, Smoothened (Smo) expression was
significantly downregulated in old mice. We used mice in which Hh signaling is specifically disrupted in myocytes (HSA Cre:Smoflox/flox) and demonstrated that Smo knockdown is sufficient to impair ischemiainduced myogenesis.
Conclusion: The present study demonstrates that Hh signaling is impaired in aged mice which leads to impaired peripheral nerve survival and myogenesis in acute ischemic stress condition. Moreover, this study brings the new concept that it is necessary to restore both peripheral nerve integrity and myogenesis in elderly to promote revascularisation of ischemic

2st Session | Sessão 2
Cell Biology and Signaling| *Biologia Celular e Sinalização*

C.06 - Sulforaphane supplementation improves vascular dysfunction in type 2 diabetes (IBILI, U Coimbra)

Speaker / *Prelector*

Cristina Sena

Abstract / *Resumo da Comunicação*

In type-2 diabetes, antioxidant depletion contributes to increased oxidative stress in the vasculature. Sulforaphane is a pleotropic molecule and a potent inducer of numerous nuclear factor erythroid-derived 2 (Nrf2)-dependent phase 2 enzymes involved in cellular response to oxidative stress. The current study was designed to assess how oxidative stress contributes to functional changes in the vasculature, and determine the importance, and the effects of pharmacologically targeting, the transcription factor Nrf2. Goto-kakizaki (GK) rats, an animal model of non-obese type 2 diabetes and age-matched control Wistar rats were treated with or without SFN during 8 weeks. At the end of the treatment, nitric oxide (NO)-dependent and independent vasorelaxation in isolated aorta and mesenteric arteries were evaluated in organ bath systems. Metabolic profile, NO bioavailability and vascular oxidative stress and Nrf2 levels were also assessed as previously (Sena et al. BJP, 2011). Diabetic GK rats presented significantly lower levels of Nrf2 ($p<0.001$) and concomitantly exhibited higher levels of oxidative stress and endothelial dysfunction (associated with decreased NO bioavailability). SFN significantly improved endothelial dysfunction and NO bioavailability. Additionally, SFN significantly decreased vascular oxidative damage (accumulation of anion superoxide and 3-nitrotyrosine in aorta and mesentery arteries, respectively). We provide experimental evidence indicating that depletion of Nrf2 in GK rats results in vascular dysfunction and sulforaphane can be used therapeutically to improve endothelial dysfunction in type 2 diabetes. All animals received care in accordance with the Portuguese Law on Experimentation with Laboratory Animals.

Work supported by: Strategic Project PEST-C/SAU/UI3282/2011-COMPETE; Project PTDC/SAU-MET/115635/2009 – COMPETE.

C.07 - Cross talk between perivascular adipose tissue and endothelial dysfunction associated with obesity (IBILI, U Coimbra)

Speaker / *Prelector*
Cristina Sena

Abstract / *Resumo da Comunicação*

Obesity is a growing problem worldwide. In this context, perivascular adipose tissue (PVAT) has recently been recognized as a novel factor in vascular biology, with implications in the pathophysiology of cardiovascular disease. PVAT is a local deposit of adipose tissue surrounding the vasculature. The main goal of this study was to investigate the effects of PVAT in endothelial function of mesenteric arteries of obese animal models. Eight-month-old male Wistar (W) rats were randomly divided in two subgroups: group 1) W control group fed with standard diet; group 2) W rats fed with high fat diet during 4 months (WHF) and compared with six-month-old male W rats. Glucose, lipids and HbA1c concentrations were measured on blood samples. Vascular contraction and functional endothelial-dependent and independent vasorelaxation was evaluated in isolated mesenteric ring preparations from the different groups in organ bath systems. Oxidative stress, inflammatory biomarkers, and adipocytokines were also evaluated in the different groups of rats by ELISA techniques. High fat diet induced significantly increased body weight, glucose at 2h and systemic levels of free fatty acids, leptin and leptin/adiponectin ratio. It also significantly reduced the efficacy of NO-dependent and independent vasorelaxation in mesenteric arteries accompanied by an increment in vascular oxidative stress. In WHF group PVAT significantly increased the expression of chemokines and pro-inflammatory adipokines, the latter of which are important contributors to endothelial dysfunction. Inflammation in PVAT directly impacts vascular disease of the underlying artery, perhaps contributing to atherosclerosis, peripheral vascular disease, hypertension, or arteriosclerosis. All animals received care in accordance with the Portuguese Law on Experimentation with Laboratory Animals.
Work supported by: Strategic Project PEST-C/SAU/UI3282/2011-COMPETE; PTDC/SAU-MET/115635/2009 – COMPETE; GAI, FMUC.

C.08 - Notch signaling in the endothelial dysfunction of systemic sclerosis

Speaker / *Prelector*

Filipe Paula

Abstract / *Resumo da Comunicação*

Motivation: Systemic sclerosis (SSc) is characterized by endothelial dysfunction, fibrosis and autoimmunity. Capillary rarefaction, dilatations, microhemorrhages, and dysangiogenesis are observed since the early stages of the disease, with high but inefficient VEGF levels. The Notch pathway is increasingly recognized as a major player in neoangiogenesis and vascular maintenance, being closely linked with VEGF-signaling . Notch activation status in the endothelium of SSc is unknown. The aim of this study is to evaluate the role of SSc-specific humoral factors in modulating Notch signaling on endothelial cells.

Methods: Confluent human umbilical vein endothelial cells were exposed for 24h to: a) VEGF-A165; b) serum from 6 Ssc patients; c) serum from 4 healthy donors. mRNA for Notch pathway components (receptors NOTCH1 [N1] and NOTCH4 [N4]; ligand DLL4 [D4]; target genes HES1, HES2 and HEY1) were measured by comparative RT-PCR using the ΔΔCt method.

Results: VEGF stimulation increased D4 (rq=2,9; p<0,001) and repressed N1 (rq=0,2; p=0,022) and HES1 (rq=0,15; p=0,023) expression. SSc serum decreased both N4 (rq=0,37; p<0,001) and D4 (rq=0,53; p=0,016), and increased HES2 (rq=1,56; p=0,020). There was a tendenc y for a reduction of N1, but it did not reach statistical significance (rq=0,43; p=0,076).

Conclusions/implications: These results suggest that Notch pathway could be activated in SSc independently of intrinsic receptor/ligand expression, perhaps inhibiting the latter retroactively. Importantly, these effects were different from those seen with VEGF stimulation, levels of which are known to be increased in SSc serum. An abnormally activated Notch pathway in the endothelium is known to produce microvascular changes that in part reproduce the SSc physiopathology. This could unravel new therapeutic targets for this yet uncurable disease.

2st Session | Sessão 2
Cell Biology and Signaling| *Biologia Celular e Sinalização*

C.09 - Elastin-derived peptides in thrombosis: friend or foe? (UMR CNRS/URCA 7369, Reims)

Speaker / *Prelector*

Pascal Maurice

Abstract / *Resumo da Comunicação*

Elastin, one of the major extracellular matrix components of the arterial wall, provides the elastic recoil properties and resilience essential for vascular function. Extracellular matrix remodeling during vascular aging is associated with elastin fragmentation and generation of soluble elastin-derived peptides (EDP) in the blood. By binding to the elastin receptor complex (ERC), our team has shown that EDP accelerate atherosclerosis in LDLR-/- and ApoE-/- mice (Gayral et al, Cardiovasc Res. 2014). However, the role of these peptides in thrombosis has been unexplored so far.

Here, we will present our recent results showing that EDP, derived from organo-alkaline hydrolysate of bovine insoluble elastin (kappa-elastin, kE), regulate thrombosis by decreasing human platelet aggregation in whole blood and washed platelets and delaying time for mesenteric arteriole occlusion in a wild-type mouse model of thrombosis induced by chemical injury (Kawecki et al, ATVB. 2014). This regulatory role of EDP on thrombosis relies on platelets expressing the ERC and on the ability of EDP to decrease plasma von Willebrand factor immobilization onto collagen. Leukocytes, mainly monocytes, are also key actors involved in thrombosis and formation of prothrombotic/procoagulant monocyte-platelet aggregates (MPA). Here, we will also present preliminary data showing that kE increases LPS-induced tissue factor (TF) expression by human monocytes both at the mRNA and protein level and thrombin generation. Our findings also suggest that EDP modulate MPA formation in whole blood by specifically decreasing the size of MPA.

In conclusion, these results demonstrate for the first time that EDP, which mainly accumulate during vascular aging, regulate thrombus formation and TF expression by monocytes. Additional studies are needed to delineate the overall outcome of these EDP in animal models related to atherothrombosis and aortic dissection wherein thrombus formation has a crucial role.

3st Session | Sessão 3

Biomaterials and Tissue Engineering | *Biomateriais e Engenharia dos Tecidos*

Chairman / *Moderador*

Brigida Bochicchio

Key-note lecture 4 - Self-renewal of Human Pluripotent Stem cells can be maintained by nanofiber composite in three-dimensional hydrogel scaffolds (U Pavia)

Livia Visai

Resumé / *Currículo Resumido*

Dr Visai is an Associate Professor in Biochemistry at the Medical Faculty of the University of Pavia. She received her degree in Biology followed by the Ph.D in Biochemistry at Pavia University.
She had been working at the University of Alabama in Birmingham and at the Center for Infectious and Inflammatory Diseases, Texas A&M University of Houston. She is Vice-Director of the Interdepartmental Center for Tissue Engineering. She received na apical position at the Salvatore Maugeri Foundation.

Abstract / *Resumo da Comunicação*

Tissue engineering evolved from the field of biomaterials development and refers to the practice of combining scaffolds, cells, and biologically active molecules into functional tissues. Recently, human pluripotent stem cells (hPSCs) constitute a promising contender as an alternative cell source in tissue engineering studies. The development of culture systems for high quality expansion and differentiation of hPSCs is still a challenging task. We report on a new type of nanocomposite scaffolds and their performance as substrates for expansion and self-renewal of hPSCs.
The gel phase of the scaffold consisted of differently crosslinked AGMA1, a guanidine-substituted
poiyamidoamine (PAA) hydrogel and the fibrous component of embedded poly-L-lactic acid (PLLA) mats of continuous electrospun nanofibers, mimicking the gel and fibrous components of tissue extracellular matrix, respectively. A non-equilibrium atmospheric pressure plasma treatment was performed to enable the adhesion between the two components.
Biological studies were performed determining the growth of human induced pluripotent stem cells (hiPSCs) or human embryonic stem cells (hESCs) seeded on the different scaffolds in comparison to cells cultured on Matrigel-coated Tissue Culture Plates as positive control. Only AGMA1-PLLA nanocomposites effectively promoted hPSC proliferation on rispect to the single components. Quantitative real-time polymerase chain reaction and immunofluorescence studies of undifferentiated markers demonstrated that the cells fully retained stemness for at least 7 days. In particular results showed that hESCs and hiPSCs cultured on AGMA1-PLLA nanocomposites expressed ali pluripotency factors analyzed. This property was absent in the two separatori components.
The potential of these or similar composites in the field of tissue engineering and regenerative medicine may be improved to specifically modulate the growth of hPSCs.

C.10 - Neoglycosylation of elastin: a novel material for regenerative medicine (U Milano-Bicocca)

Speaker / *Prelector*

Gabriele Corsaro

Abstract / *Resumo da Comunicação*

A way to restore the functional role of damaged tissues is based on their substitution with biomimetic matrices able to replicate the appropriate role of Extracellular Matrix (ECM) in the regulation of cell microenvironment. One of the main components of ECM, elastin, plays a leading role in wound healing because of its smart behavior in terms of structural plasticity and mechanical stability.
The design of elastin matrices that can be functionalized with signaling molecules in order to upgrade elastin to a cell-responsive biomaterial, without altering its structural features is of primary relevance in order to preserve its native recognition and biomolecular properties.
A bioconjugation approach on elastin has been exploited for the immobilization of selected carbohydrate epitopes. Carbohydrates are interesting biomolecules for material functionalization because of their polyhydroxylated nature, that confers hydrophilic features to the surface they are linked to, and for their ability to convey biological information.
The chemical neoglycosylation did not alter the morphology of the protein, as assessed by SEM and solid-state FTIR.
Preliminary biological evaluation will be presented.
Acknowledgements.
The work has been supported by Fondazione Cariplo grant n° 2011-0270, and PRIN 2011-2010L9SH3K

C.11 - Removal of alpha-Gal xenogenic barrier from bioprosthetic heart valve substitutes for improvement in cardiopathic patients life length and quality (U Padova)

Speaker / *Prelector*

Michel Spina

Abstract / *Resumo da Comunicação*

Xenogeneic tissues are widely employed in cardiac surgery although in absence of any assessment of xenogeneic material elimination. Adopted glutaraldehyde treatments are unable to grant a complete immuno-tolerance by reducing but not eliminating the immunogenicity particularly for the alpha-Gal epitope. The primary cause of failure of bioprosthetic heart valves is dystrophic calcification, strongly related to the inflammatory/immune reactions elicited by the exposed xenoepitopes. Every year, 300.000 patients are receiving bioprosthetic heart valves or pericardial patches of bovine, porcine or equine origin. Heart valve replacement constitutes a major healthcare problem worldwide (the second most frequent cardiac surgery procedure). Bioprosthetic heart valves suffer from late dysfunction restricting their successful application to older recipients. Moreover re-interventions weigh heavily on the economic and social aspects, undermining the quality of life of the patient. We have developed an easy and efficient procedure allowing to remove the alpha-Gal xenoantigen. The procedure has been devised in order to process bioprosthetic heart valves already on the market (Magna model and Edwards Lifesciences) and equine pericardial patches (XAG-400 model Edwards Lifesciences). The amount of alpha-Gal epitopes before treatment (as number of epitopes/10 mg of wet tissue) resulted in 5.21 ± 0.7*1010 and 9.7 4.9*1010, respectively. After treatment the alpha-Gal antigens were completely removed also confirmed by successive immunofluorescence analysis. Additionally the in-vitro amount and speed of thrombin formation, as well as the complement activation degree were much lower in the processed heart valve bioprostheses than in the same untreated biomaterials. A significant potentiality of this treatment is its application to bioprostheses ready-to-sell, without introducing any major change in the production process, while ensuring containment of the industrial production costs.

3st Session | Sessão 3
Biomaterials and Tissue Engineering | *Biomateriais e Engenharia dos Tecidos*

C.12 - Elastogenesis in a tissue-engineered heart valve specifically developed for the mitral position (RWTH Aachen University)

Speaker / *Prelector*

Ricardo Moreira

Abstract / *Resumo da Comunicação*

Elastic fibres are crucial components of cardiovascular tissues such as blood vessels and heart valves providing recoil after stretching during each cardiac cycle for a life-time. It is well known that tissue-engineered heart valves lack in vitro elastin formation and, therefore, their long-term functionality after implantation might be limited. We have recently demonstrated the first tissue-engineered mitral valve – TexMi – in which elastin formation was observed after 25 days of in vitro cultivation. This is an exciting result as no elastogenesis was reported before in tissue-engineered heart valves neither by our group nor by others. The valve recapitulates the anatomical components of the native one (annulus, asymmetric leaflets and chordae tendineae) and is based on a hybrid scaffold composed of fibrin gel as cell carrier and a warp-knitted textile mesh. Human umbilical cord veins were used as cell source. The valves were fabricated by injection moulding and cultivated under dynamic conditions. Valvular functionality was assessed in a custom-made flow-loop circulation system according to ISO standards. Tissue analysis included histology, immunohistochemistry, transmission electron microscopy, two-photon scanning microscopy, biochemical assays, burst strength measurements and tensile tests. The valves (n=3, diameter = 2.4 cm) revealed an excellent hydrodynamic performance with an effective orifice area of 2.49 ± 0.15 cm2, regurgitation of 4.3 ± 0.4 % and mean gradient pressure of 2.0 ± 1.2 mmHg. Tissue samples from different parts of the valves burst at 845-962 mmHg and Young`s moduli were 0.12-0.60 MPa. Tissue analysis showed abundant collagen, glycosaminoglycans and elastin. Elastogenesis was detected already after one week of dynamic cultivation with a steady increase in the following cultivation period. All in all, these are encouraging results towards the fabrication of a fully functional, viable prosthesis to be implanted in the mitral position.

Key-note lecture 5 - Bi-layer polyurethane-extracellular matrix cardiac patch improves ischemic ventricular wall remodelling in a rat model (Pittsburgh U)

Antonio D'Amore

Resumé / *Currículo Resumido*

Antonio D'Amore, MSc in Mechanical Engineering at University of Palermo (Italy), MSc in Biomedical Engineering at Imperial College London (UK), PhD in Tissue Engineering at the University of Palermo & University of Pittsburgh (USA). Research associate and RiMED Fellow at the McGowan Institute for Regenerative Medicine, University of Pittsburgh. Dr D'Amore' research activity focuses on the development of tissue engineering paradigms and biomaterials for cardiovascular tissue regeneration.

Abstract / *Resumo da Comunicação*

The American Heart Association 2014 update estimates 2% of the total US population is affected by congestive heart failure (CHF). Left ventricle (LV) pathological remodeling is a compensatory response initiated in CHF. Approaches developed to mitigate LV remodeling include drug treatments, surgical procedures, and restraint devices. While these approaches have met with success in some patient populations, progression towards end-stage heart failure still occurs. Restraint devices, meant to provide a permanent physical block towards gross dilatation of the heart, have not progressed to clinical adoption. A promising alternative is to employ a biodegradable cardiac patch. In this approach, a scaffold is applied to the epicardial surface to provide temporary, local, and mechanical support and impact the remodeling process to help sustain LV function. The question arises as how to design such a patch to provide appropriate mechanical support and also to stimulate a positive remodeling. We proposed a bi-layered cardiac patch composed of a biodegradable poly-ester carbonate urethane-urea, (PECUU) enriched layer providing mechanical support, and a porcine cardiac extracellular matrix rich (cECM) layer, providing an environment conducive for host cell recruitment. The effects of the cECM-polymer bi-layer patch have been assessed in vivo on a rat infarction model. Three patch types have been studied: PECUU patch with stiffer direction aligned with the heart circumferential direction, PECUU patch with stiffer direction aligned with the heart longitudinal direction, PECUU-cECM isotropic patch. Scaffolds have been implanted at 2 weeks after a left coronary artery ligation procedure. Explants analysis conducted at 8 weeks from the implantation showed the capacity of the bi-layer cardiac patch to significantly (1) mitigate LV wall thinning, (2) reduce scar formation, (3) promote host cell infiltration, (4) prevent ventricle dilation, (5) improve cardiac function.

4st Session | Sessão 4

Innovation and Technology from Diagnostics to Therapeutics | Inovação *e Tecnologia de Diagnóstico à Terapêutica*

Chairman / *Moderador*

Philippe Charpiot

Key-note lecture 6 - SClinical and experimental potential of the noninvasive quantification of myocardial perfusion entropy by nuclear imaging (U Grenoble)

Laurent Riou

Resumé / *Currículo Resumido*

Laurent Riou is a research scientist within the French Institute of Health & Medical Research (INSERM) in the laboratory Radiopharmaceutiques Biocliniques currently headed by Pr. C. Ghezzi in Grenoble, France. His research interests are mainly focused on the preclinical and clinical development of nuclear imaging for the early diagnosis and management of cardiovascular diseases, with additional basic science interests in the biomechanics of atherosclerotic plaques and more applied research interests in the field of drug or tracer vectorization

Abstract / *Resumo da Comunicação*

Cardiovascular (CV) diseases represent the leading cause of mortality worldwide. CV diseases are mainly caused by coronary heart disease. Major CV risk factors include hypertension, hypercholesterolemia, obesity and diabetes. In some populations, the proportion of patients with ≥1 cardiovascular risk factor may reach 80%. From a pathophysiological perspective, cardiovascular risk factors lead to an initially asymptomatic microvascular dysfunction (MVD) characterized by capillary loss and endothelial dysfunction according to mechanisms that remain to be fully determined. MVD persist in the later stages of coronary heart disease evolution in parallel with the development of vulnerable coronary atherosclerotic plaques. Invasive methods allow the clinical identification of MVD through the determination of the index of microcirculatory resistance (IMR). IMR has demonstrated significant prognostic value but its use remains limited due to the required invasive methodology as mentioned above.
The nuclear imaging of myocardial perfusion is routinely performed clinically for the assessment of cardiovascular prognosis. The hypotheses developed in this presentation will be that (1) the quantification of entropy from clinical scintigraphic images of myocardial perfusion might reflect myocardial perfusion heterogeneity and therefore MVD caused by cardiovascular risk factors and might therefore have independent and incremental prognostic value, and (2) the determination of myocardial perfusion entropy on small animal models of cardiovascular diseases might greatly help to characterize the mechanisms implicated in early microcirculatory dysfunction.

C.13 - Hemorheology and Vascular Diseases (IMM, U Lisboa)

Speaker / *Prelector*

Carlota Saldanha

Abstract / *Resumo da Comunicação*

A review about (i) blood and vessels composition, properties and functions, (ii) hemorheology and (iii) inflammation meaning will be present.
Clinical evidence between the interplay of hemorheology and micro and macro vascular diseases will be review.

C.14 - Galectin 3 levels after running – a systematic review and perspective (Hospital Center of Vila Nova de Gaia/Espinho)

Speaker / *Prelector*

Eduardo Vilela

Abstract / *Resumo da Comunicação*

Motivation: Running is a physiologic human activity. Galectin 3, a mediator in the development of cardiac fibrosis, is a promising biomarker in heart failure. Reports demonstrate that cardiac biomarkers, such as high sensitivity cardiac troponin (hs-cTn), can increase substancially after running, but the significance of this data is controversial. As a marker of fibrotic burden, galectin 3 could help explain part of the physiologic basis for this phenomenon. We aimed to review the evidence concerning galectin 3 levels after running. Methods: A literature search was conducted on three databases (Pubmed, ISI and Scopus) up to March 2015. The queries used were "galectin 3 AND running" and "galectin 3 AND exercise". Additional records were identified through review of the literature. It was mandatory for the article to specify the upper reference level (URL) used for galectin 3, and the distance covered. Studies written in languages other than English were excluded. Studies selecting participants based on a specific pathology were outside the scope of this review, which intended to describe a healthy (or presumably healthy) population. Results: A total of 68 articles were found, and of those 2 fulfilled inclusion criteria(1-2). A total of 39 runners were assessed (21 in a 30 km run, 18 in a 60 km run). Galectin 3 levels exceed the URL in 28 (71.8%) runners. There was no correlation between galectin 3 and hs-cTn. Conclusion: Galectin 3 levels increased above URL in 71.8% of runners. Values were not correlated to hs-cTn, suggesting different mechanistic pathways. More studies, and a larger number of subjects, are needed in order to clarify these results, and its possible cardiac consequences.
1 - Salvagno GL, et al. The concentration of high-sensitivity troponin I, galectin-3 and NT-proBNP substantially increase after a 60-km ultramarathon. Clin Chem Lab Med 2014;52:267-72
2 - Hättasch R, et al. Galectin-3 increase in endurance athletes. Eur J Prev Cardiol. 2014;21:1192-9

4st Session | Sessão 4

Innovation and Technology from Diagnostics to Therapeutics | Inovação *e Tecnologia de Diagnóstico à Terapêutica*

C.15 - Physiological computing: using vascular signals for something completely different (IBEB, U Lisboa)

Speaker / *Prelector*

Hugo Ferreira

Abstract / *Resumo da Comunicação*

In this communication I will present the field of physiological computing (PC), a recent biomedical endeavor. We will discuss the principles of PC, including the concepts of human-computer interface (HCI) and biofeedback. We will see how to build such a HCI and use vascular signals to interact with a computer or device. In particular, we will see how these interfaces can be used for rehabilitation, arts or just for fun!.

Key-note lecture 7 - Opportunities for Direct Digital Manufacturing in Vascular Research (PU Leiria, CDRSP)

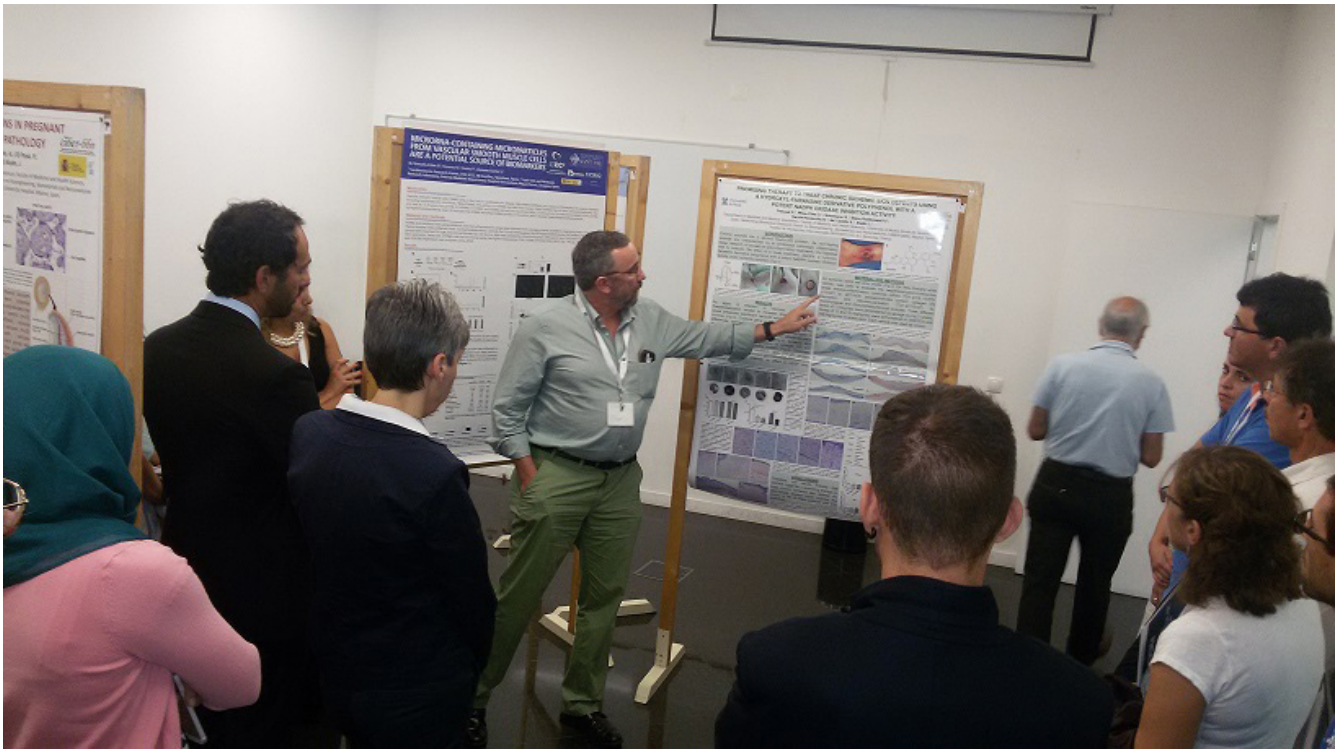
Geoffrey Mitchell

Resumé / *Currículo Resumido*

Professor Geoffrey Mitchell is the vice-director of the Centre for Rapid and Sustainable Product Development of the Institute Polytechnic Leiria. Previously he was Professor Polymer Physics and Director of the Centre for Advanced Microscopy at the University of Reading. His current research is focused on the development of new materials and processes for Direct Digital Manufacturing and in particular materials which bring function to devices prepared using Direct Digital Manufacturing. He has recently edited a book ´Electrospinnning: principles, practice and possibilities

Abstract / *Resumo da Comunicação*

Direct Digital Manufacturing is a family of technologies which are able to manufacture an object directly from a digital definition without the need for specific moulds or tools. Direct Digital Manufacturing provides a route to mass customization and this is particularly suited for the production of medically related devices. We review the opportunities for the use of these new technologies for the development of implants for use in vascular systems. An early use of selective laser melting techniques has been the production of personalized stent-like devices. Another area of intense interest is the use of Direct Digital Manufacturing techniques for the preparation of scaffolds for tissue engineering in which the development of a vascular network is critical for cell proliferation. One aspect of Direct Digital Manufacturing which once appeared impossible is the concept of whole organ production. Recent work has shown that it is possible to produce through tissue engineering the complex vascular structure typical of an organ. We critical review a number of routes of achieving this and propose some alternatives. One promising route is to use fibres coated with endothelial cells. These are then covered with a protein based material, rich in cells. The material infused between the fibres was cross-linked with light which allowed the coated fibres to be removed. This left a delicate network of tiny spaces throughout the cross-linked cell material. The human endothelial cells which were also still in the matrix materials proliferated and developed in to stable capillaries. The development of such complex vascular network makes the concept of whole organ manufacturing a step closer and we use this approach to identify alternative multistage proceses and identify the key technological developments required.



5st Session | Sessão 5
Clinical Applications | Aplicações Clínicas

Chairman / *Moderador*

Vicenta Llorente-Cortés

C.16 - Identification of differentially expressed proteins in atherosclerotic patients with type 2 diabetes (U Sassari)

Speaker / *Prelector*

Antonio Leppeda

Abstract / *Resumo da Comunicação*

Atherosclerosis is a form of chronic inflammation characterized by the accumulation of lipids and fibrous elements in medium and large arteries that represents a major cause of death and disability in people with diabetes.
The aim of this study is to identify differentially expressed plasma proteins between patients with or without type 2 diabetes undergoing carotid endarterectomy, by applying two-dimensional electrophoresis analysis coupled with mass spectrometry.
Briefly, 14 plasma samples from diabetic patients and 15 plasma samples from non diabetic patients were subjected to a low-abundance proteins enrichment step using hexapeptide combinatorial ligand libraries (ProteoMiner™ enrichment kit, Bio-Rad Laboratories) followed by two-dimensional electrophoresis. This analytical tecnique allows resolving hundred of different protein isoforms according to both isoelectric point and molecular weight. Protein profiles were compared by using PD-Quest software (Bio-Rad Laboratories).
Preliminary results show a panel of proteins differentially expressed between the two groups of atherosclerotic patients. Their identification by peptide mass fingerprinting analysis using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) is in progress. Actually, identification of markers in diabetic patients could be of interest for clarifing the biochemical mechanisms underlying the strong association between diabetes and atherosclerosis.
This work was supported by Regione Autonoma della Sardegna (Legge Regionale 7 Agosto 2007, N.7 – Bando 2010 - Grant no. CRP-26789 and P.O.R. Sardegna F.S.E. 2007/2013, Asse IV Capitale Umano - Obiettivo competitività regionale e occupazione, Asse IV Capitale umano, Linea di Attività l.3.1) and by Fondazione Banco di Sardegna.

C.17 - Comparing the oscillatory properties of the LDF signal recorded from subjects with different ages during a passive leg raising test

Speaker / *Prelector*

Henrique Silva

Abstract / *Resumo da Comunicação*

Skin microcirculation suffers morphological and functional changes with ageing, which can be assessed in vivo with laser Doppler flowmetry (LDF). Provocations tests are used to increase the sensitivity of such quantifications by allowing the assessment of regulatory mechanisms. The passive leg raising (PLR) is a classic maneuver to assess peripheral microcirculatory function. Our aim was to compare the LDF signal obtained from subjects of different ages during PLR, with several analysis tools: the wavelet transform, which fractions the signal into its main frequency components (heart, respiration, myogenic, sympathetic, endothelial); detrended fluctuation analysis (DFA), which assesses its correlation properties through the calculation of an alpha exponent; and the multiscale entropy analysis (MSE), which quantifies the complexity of the signal. 59 subjects enrolled on this study after informed consent and were divided into two groups according to age – group 1 (N=35; 18-35 y o; mean age=22.3) and group 2 (N=24; 40-65 y o; mean age=). The LDF signal was recorded on the inferior aspect of the second toe. Subjects performed a PLR protocol consisting of three phases: baseline, provocation and recovery. The Mann Whitney test for independent samples was used for group comparison ($p<0.05$). Older subjects showed significantly lower respiratory and higher myogenic activities during baseline. During provocation, lower respiratory and endothelial activities together with higher myogenic and sympathetic activities were also found for these subjects. These also showed a significantly lower respiratory alpha exponent and a significantly higher myogenic complexity during provocation. These results suggest that our experimental approach is sensitive to detect age-related changes in the in vivo peripheral microvascular function.

5st Session | Sessão 5
Clinical Applications | Aplicações Clínicas

C.18 - Endothelium-dependent vasoactivity of the human internal mammary artery: the influence of cardiovascular risk factors (FF, U Coimbra)

Speaker / *Prelector*

Diogo Fonseca

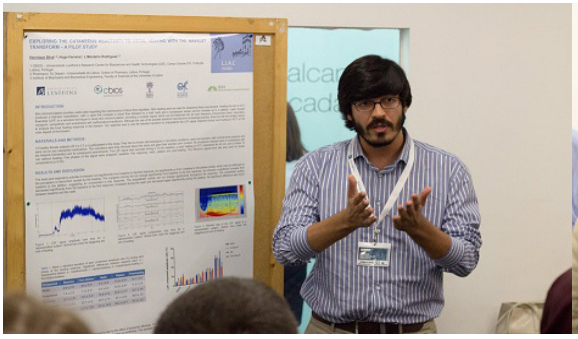
Abstract / *Resumo da Comunicação*

The human internal mammary artery (HIMA) has long been considered the best graft to use in coronary artery bypass grafting (CABG) due to several advantages of this graft compared to others, i.e. the lower incidence of atherosclerosis and vasospasm. This work aimed at studying the endothelial function of the HIMA in the presence of several cardiovascular risk factors (modifiable and non-modifiable) and concomitant diseases.

The discarded segments of HIMA were harvested from 40 patients (age between 47 and 81) undergoing CABG. All the experiments were performed with the approval by the Ethics Committee of Coimbra University Hospitals (reference PC-388/08) and the clinical data was collected with the approval by the Ethics Committee of Faculty of Medicine, University of Coimbra (reference 107/2014). The relaxation was evaluated using acetylcholine (ACh) and sodium nitroprusside (SNP) after pre-contraction with noradrenaline (NA, 1 μM). Univariate and multivariate statistical analysis was applied.

The sample included patients with several modifiable cardiovascular risk factors (hypertension, smoking history, diabetes, dyslipidemia, among others). The results showed that the vasorelaxation is generally lower in patients who present one or more modifiable cardiovascular risk factors. Results also showed that the relaxation induced by SNP was about 3 times higher than the relaxation to ACh. Additionally, the results demonstrated there is a moderate to strong correlation between gender and the relaxation both to ACh and SNP, with a higher vasodilation in male patients.

In conclusion, patients who require coronary revascularization usually present multiple risk factors and other diseases which can interfere with several pathways of regulation of the endothelial function. This study suggested that the cardiovascular risk profile influences the endothelium-dependent vasoactivity. Further studies are required to establish a stronger correlation with the several risk factors.



Panel Comunications | *Comunicações em Posters*

Abstract List | *Lista de Resumos Submetidos*

P.01 **DENDRITIC CELLS TAKE PART TO EARLY AND LATE RESPONSE TO ISCHEMIC MYOCARDIAL INJURY**. Paolo Romagnoli, Laura Pieri, Stefano Bacci, Beatrice Defraia, Gian Aristide Norelli, Aurelio Bonelli

P.02 **MICRORNA-CONTAINING MICROPARTICLES FROM VASCULAR SMOOTH MUSCLE CELLS ARE A POTENTIAL SOURCE OF BIOMARKERS.** David de Gonzalo-Calvo, Ana Cenarro, Fernando Civeira F; Vicenta Llorente-Cortés.

P.03 **LASER DOPPLER FLOWMETRY (LDF) AS AN ALTERNATIVE TECHNIQUE TO EVALUATE PERFUSION RECOVERY ON A MURINE MODEL OF HIND LIMB ISCHEMIA**. Henrique Silva, Alain-Pierre Gadeau, Marie-Ange Renault, Hugo Ferreira, M Julia Bujan, L Monteiro Rodrigues.

P.04 **EXPLORING THE CUTANEOUS REACTIVITY TO LOCAL HEATING WITH THE WAVELET TRANSFORM – A PILOT STUDY**. Henrique Silva, Hugo Ferreira, L Monteiro Rodrigues.

P.05 **CRYOPRESERVATION OF BIOENGINEERED, DECELLULARIZED AORTIC VALVES DOES NOT COMPROMISE THEIR PROPENSITY TO REPOPULATION BY HOST CELLS AND TISSUE REMODELLING. GOOD AND LESS GOOD OUTCOMES**. Antonella Bonetti, Michele Gallo, Filippo Naso, Paolo Franci, Adolfo Paolin, Roberto Busetto, Michele Spina, Gino Gerosa, Fulvia Ortolani.

P.06 **PROMISING THERAPY TO TREAT CHRONIC ISCHEMIC SKIN DEFECTS USING A HYDROXYL-FARNASINE DERIVATIVE POLYPHENOL WITH A POTENT NADPH OXIDASE INHIBITION ACTIVITY.** Pascual G., Mesa-Ciller C., Sotomayor S., Blanc-Guillemaud V., García-Honduvilla N., del Castillo E., Buján J.

P.07 **PLACENTAL STRUCTURAL ALTERATIONS IN PREGNANT WOMEN WITH VARICOSE VEIN PATHOLOGY.** Mesa-Ciller, C.; Álvarez-Rocha, MJ.; Asúnsolo, A.; Payá, P.; Cifuentes, A.; García-Honduvilla, N.; Buján, J.

P.08 **EXPLORING THE EFFECT OF POSTURE ON THE PERIPHERAL CUTANEOUS MICROVASCULAR RESPONSE TO THE TOPICAL APPLICATION OF METHYL NICOTINATE.** Henrique Silva, Catarina Rosado, Hugo Ferreira, Joana Antunes and L Monteiro Rodrigues.

P.09 **NAILFOLD CAPILLAROSCOPY CRITERIA FOR DISTINCTION OF PRIMARY AND SECONDARY RAYNAUD'S PHENOMENON.** Marta C. Amaral, Filipe S. Paula, Joana Caetano,Isabel A. Ferreira, Susana Oliveira1, J. Delgado Alves.

P.010 **APPLICATION OF PHOTOPLETHYSMOGRAPHY TO MONITOR HEART RATE**. João Requicha, Clemente Rocha, Rui Assunção, Henrique Silva, Margarida Estudante, Luis Lobo, Luís Monteiro Rodrigues.

P.011 **DIFFERENT UPTAKE OF NATIVE VERSUS AGGREGATED LDL BY AORTIC VALVE INTERSTITIAL CELLS IN NORMOLIPIDEMIC-LIKE IN-VITRO CONDITIONS**. Antonella Bonetti, Vicenta Llorente-Cortés, Alberto Della Mora, Magali Contin, Franco Tubaro, Maurizio Marchini, Fulvia Ortolani.

P.012 **TESTING TISSUE PROTECTIVE CAPACITIES OF PLANT EXTRACTS**. Patrícia Rijo, Marisa Nicolai, Ana Sofia Fernandes, Luís M. Rodrigues.

P1 - Dendritic cells take part to early and late response to ischemic myocardial injury

Paolo Romagnoli[1], Laura Pieri[2], Stefano Bacci[1], Beatrice Defraia[2], Gian Aristide Norelli[2], Aurelio Bonelli

[1]Department of Experimental and Clinical Medicine
[2]Departement of Health Sciences, University of Florence,Italy

Although heart infarction is a major cause of death and disability worldwide, the comprehension of heart tissue response to injury is still limited. Since dendritic cells are involved in the regulation of immune processes and take part to inflammatory cell infiltrates, even in vascular wall, they may be candidate to a pivot role in that response. Other possible candidates are mast cells, which are suspected to react quickly to myocardial ischemia, but histological evidence for these cells is contradictory.
Samples of left ventricle were taken at autopsy from subjects deceased from acute myocardial infarction with symptoms onset since less that 6 h, or from trauma; the latter had either evidence of previous infarction, or of coronary artery disease without infarction, or no evidence of coronary artery and myocardium disease (controls). The study complied with Italian law and Helsinki declaration. Methods included bulk staining with triphenyltetrazolium, histological staining with routine methods and immunolabelling for markers of dendritic cells, mast cells and granulocytes.
Triphenyltetrazolium test was positive in acute infarction only if autopsy was performed within 48 h from death. Significant infiltration ($p<0.05$ vs. controls) of dendritic cells was found in acute infarction and in cases of previous infarction, the latters also showed significant amounts of granulocytes. Mast cells numbers and granule content were always similar to controls.
These results suggest that dendritic cells exhibit early and protracted reaction to myocardial ischemic injury and therefore may be candidate regulators of inflammatory and scarring responses in this tissue, which would make them possible targets to control those processes, and markers of acute heart infarction, which would be of use in forensic medicine assessment of sudden death cases.

P2 - MicroRNA-containing microparticles from vascular smooth muscle cells are a potential source of biomarkers

David de Gonzalo-Calvo[1], Ana Cenarro[2], Fernando Civeira F[2]; Vicenta Llorente-Cortés, PhD[1]

[1]Cardiovascular Research Center, CSIC-ICCC, IIB-SantPau, Barcelona, Spain.
[2]Lipid Unit and Molecular Research Laboratory, IIS Aragón, Hospital Universitario Miguel Servet, Zaragoza, Spain.

Motivation: We tested whether vascular smooth muscle cells (VSMC)-derived microparticles (MP) contain extracellular microRNAs (miRs) and the potential value of these miRs as biomarkers of cardiovascular disease.
Methods: Human VSMC and explants from atherosclerotic or non-atherosclerotic areas were obtained from coronary arteries of patients undergoing heart transplant. Plasma samples were collected from: normocholesterolemic controls (N=15) and heterozygous familial hypercholesterolemia (FH) patients (N=15). Both groups were matched for age, sex and cardiovascular risk factors. MP were isolated by ultracentrifugation. MP size (0.1 μm-1 μm) was analyzed using flow cytometry. Detection of RNAs was performed by using the Agilent Bioanalyzer 2100. VSMC-enriched miRs (miR-21-5p, -143-3p, -145-5p, -221-3p and -222-3p) expression was analyzed using qPCR.
Results: Exposition of VSMC to conditions mimicking hypercholesterolemia induced a decrease in miR-143-3p and -222-3p in MPs. MP derived from atherosclerotic plaque areas showed a decreased level of miR-143-3p and -222-3p compared to non-atherosclerotic areas. miR-222-3p levels were lower in circulating MP from familiar FH patients compared to normocholesterolemic controls.
Conclusions: MP secreted by human coronary VSMC contain miRs. Hypercholesterolemia alters the miR profile of VSMC-derived MP. The miR content of VSMC-derived MP is a potential source of cardiovascular biomarkers.

P3 - Laser Doppler Flowmetry (LDF) as an alternative technique to evaluate perfusion recovery on a murine model of hind limb ischemia

Henrique Silva[1,2], Alain-Pierre Gadeau[3,4], Marie-Ange Renault[3,4], Hugo Ferreira[5], M Julia Bujan[6], L Monteiro Rodrigues[1,2]

[1]CBiOS – Universidade Lusófona's Research Center for Biosciences and Health Technologies (UDE), Campo Grande 376, 1749-024, Lisboa, Portugal
[2]Pharmacol. Sc Depart – Universidade de Lisboa, School of Pharmacy, Lisboa, Portugal
[3]Université de Bordeaux, Adaptation cardiovasculaire à l'ischémie, U1034, F-33600 Pessac, France
[4]INSERM, U1034, Adaptation cardiovasculaire à l'ischémie, F-33600 Pessac, France
[5]Institute of Biophysics and Biomedical Engineering, Faculty of Sciences of the University of Lisbon
[6]Faculty of Medicine, Alcalá de Henares, Madrid, Spain

Peripheral arterial disease (PAD) is a complex vascular disease, whose pathophysiological process is not fully understood. It is of difficult to study in patients due to its slowly progressing nature. To help investigate the mechanisms underlying PAD pathogenesis, several rodent models of hind limb ischemia are available, in which Laser Doppler Imaging (LDI) is a reference technique in assessing perfusion recovery. Laser Doppler Flowmetry (LDF), however, despite its wide use in microvascular assessment, is not commonly used for this particular purpose.
Our objective was to evaluate the peripheral microcirculatory recovery on a murine model of unilateral hind limb ischemia with both LDI and LDF.
Ischemia was induced on the left hind limb of nine male C57BL/6 mice (24.4 ± 2.0 g, 11 weeks old) while under isoflurane anesthesia (5% for induction, 3% for maintenance), by ligation and total excision of the superficial femoral artery. The contralateral hindlimb served as control.
For the vascular assessments, the animals were anesthetized with an intraperitoneal administration of ketamine (125 mg/kg) and xylazine (10 mg/kg). Microcirculatory perfusion of the feet was then assessed with LDI and LDF before and on postoperative days 4, 6, 9, 12, 15 and 21. Calculated perfusion was expressed as the ratio of ischemic (left) to nonischemic (right) LDI and LDF values. The Wilcoxon signed-rank test was used for statistical comparisons ($p<0.05$). LDI and LDF perfusions values were tested for correlation using Spearman's rho coefficient.
No perfusion differences between the feet were found with LDI or LDF before surgery. Both LDI and LDF ratios were significantly reduced on postoperative days 4, 6, 9, 12 and 16 relative to the one before surgery, but no differences were found on day 21. LDF and LDI perfusion values were shown to be significantly correlated throughout the evaluation period.
These results suggest a potential use of LDF as an alternative technique to LDI to evaluate the microcirculation perfusion recovery on hindlimb ischemia murine models. Although it measures a smaller area of tissue compared to LDI, LDF has the advantage of allowing real-time recordings, which can give informations of the perfusion dynamics during provocation tests. Furthermore, the LDF signal can be decomposed into its main activity components, providing insight of the underlying regulatory mechanisms..

P4 - Exploring the cutaneous reactivity to local heating with the wavelet transform – a pilot study

Henrique Silva[1,2], Hugo Ferreira[3], L Monteiro Rodrigues[1,2]

[1]CBiOS – Universidade Lusófona's Research Center for Biosciences and Health Technologies (UDE), Campo Grande 376, 1749-024, Lisboa, Portugal
[2]Pharmacol. Sc Depart – Universidade de Lisboa, School of Pharmacy, Lisboa, Portugal
[3]Institute of Biophysics and Biomedical Engineering, Faculty of Sciences of the University of Lisbon

Skin microcirculation provides useful data regarding the mechanisms of blood flow regulation. Skin heating tests are used for assessing these mechanisms. Heating the skin at 42°C produces a biphasic vasodilation, with a rapid first increase in blood flow, followed by a brief nadir and a subsequent slower second increase up to a plateau. Laser Doppler flowmetry (LDF) is a sensitive technique to study skin microcirculation, providing a complex signal, which can be fractioned into its main frequency components (heart, respiration, myogenic, sympathetic and endothelial) with the wavelet transform. Our objective was to characterize the LDF signal obtained during a local skin heating protocol with the wavelet transform. Local blood flow was recorded on the volar aspect of the forearm of 10 healthy female subjects (25.0 ± 3.7 y o). The LDF signal was recorded during a 10 min baseline, a 40 min skin heating at 42°C, and a further 10 min without heating. Five phases of the signal were analyzed: baseline, first response, nadir, plateau and post-heating. The Wilcoxon signed-rank test was used for phase comparisons ($p<0.05$). The heart, respiratory and myogenic activities increased non-significantly from baseline to the first response, but significantly so to plateau phase; first response and plateau gave significantly different responses. Sympathetic activity did not change significantly between the different phases of the protocol. Endothelial activity significantly decreased from baseline to the first response and to plateau. This activity was also found to be significantly higher for the first response. The sensorial afferents, the sympathetic nervous system and the endothelial NO release are known to play a role in this response. Our data seems to suggest that a change in myogenic activity is also involved. The wavelet transform seems to be a suitable tool to characterize the LDF signal recorded during local skin heating in regards to the mechanisms involved.

P5 - Cryopreservation of bioengineered, decellularized aortic valves does not compromise their propensity to repopulation by host cells and tissue remodelling. Good and less good outcomes

Antonella Bonetti[1], Michele Gallo[2], Filippo Naso[2], Paolo Franci[3], Adolfo Paolin[5], Roberto Busetto[3], Michele Spina[4], Gino Gerosa[2], Fulvia Ortolani[1]

[1]Department of Experimental and Clinical Medicine, University of Udine, 33100, Udine, Italy
[2]Division of Cardiac Surgery, Department of Cardiac, Thoracic and Vascular Sciences, University of Padua, 35122, Padova, Italy
[3]Department of Animal Medicine, Productions and Health, University of Padua, 35020, Legnaro, Italy
[4]Department of Biomedical Sciences, University of Padua, 35122, Padova, Italy
[5]Tissue Bank of Veneto Region, Treviso Regional Hospital, 31100, Treviso, Italy

In the last two decades, clinical demand of allogeneic valve substitutes exceeded cryobank supply of human heart valves, despite their long-term failure due to host-versus-graft immune responses. Previously, TRICOL-decellularized porcine aortic valve allografts (TDVs) implanted in Vietnamese pigs showed functional capacity besides being permissive of in-vivo spontaneous repopulation by host cells and tissue remodelling. Here, using the same animal model, TDVs were compared with others additionally subjected to cryopreservation/thawing (TDCVs). Animal use for experimental purposes was authorized by the Italian Ministry of Health (27/08 C16 project), according to D.L. n. 116, art. 12, January 27, 1992. All procedures on animals were performed in compliance with ISO 10993-1, ISO 10993-2, and UNI EN ISO 5840 standards. For both allograft types, almost complete re-endothelialization was observed as well as repopulation by cells resembling fibroblasts, myofibroblasts, and smooth muscle cells, some of which exhibited typical canals of collagen fibrillogenesis and elastogenesis-related features revealing tissue remodelling occurrence. Moreover, neo-vascularization and re-innervation involved aorta medial and adventitial tunicae. Altered regions were also apparent, which showed tissue lesions including calcification, affecting TDCVs more severely than TDVs. In conclusion, cryopreservation was found to limit the favourable outcomes characterizing TDVs, although hemodynamic performance and permissivity to cell repopulation and tissue renewal were not compromised. Thus, these preclinical data suggest potential feasibility of cryobank-derived hemodynamically functional and self-regenerating allogeneic valve substitutes, once optimization of pre-implantation procedures or their mutual compatibility will be accomplished.

P6 - Promising therapy to treat chronic ischemic skin defects using a hydroxyl-farnasine derivative polyphenol with a potent nadph oxidase inhibition activity

Pascual G.[1], Mesa-Ciller C.[1], Sotomayor S.[1], Blanc-Guillemaud V.[2], García-Honduvilla N.[1], del Castillo E.[1], Buján J.[1]

[1]Department of Medicine and Medical Specialities. Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain. Networking Biomedical Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.
[2]Institut de Recherches Internationales SERVIER (I.R.I.S.), Suresnes, France.

Introduction: Chronic wounds are a serious healthcare problem. As non-healing wounds are characterized by a continuous pathologic inflammatory stage, research is focused on anti-inflammatory treatments. Our objective was to analyze, the effect of a novel treatment, S42909, a hydroxyl-farnasine derivative polyphenol with a potent NADPH oxidase inhibition activity under ischemic condition.
Material and methods: An ischemic rabbit ear ulcer model (24 New Zealand white rabbits) was used to evaluate the reepithelialization/contraction areas, anti/pro-inflammatory cytokines mRNA (TGF-β1/IL-10/IFN-γ/VEGF) by qRT-PCR, collagen I/III deposition and neovascularization (TGF- β1/VEGF) by morphological and immunohistochemical analysis. Three different doses of 1 ml/kg/day were administered by gavage during 2 weeks. Doses of 10 and 30 mg/kg/day were administered in SMEDDS3 and 100 mg/kg/day in arabic gum. Each vehicle was used as control.
Results: No signs of infection or necrosis were found. Reepithelialization tended to increase dose-dependent. All doses presented significant reduced wound contraction than controls. Cytokine gene expression did not displayed differences among doses used. Dose of 100 mg/kg/day displayed a significant increase of TGF-β1 mRNA compare to control and 30 mg/kg/day of IL-10 mRNA. No differences were observed in INF-γ and VEGF mRNA. Ischemic skin wound areas had scarce expression of collagen I/III and showed rich glycosaminoglycans content. Treatment increased the collagen deposition and TGF-β1 protein expression, and decreased glycosaminoglycans content dose-dependent; however no effect in VEGF was appreciated.
Conclusions: Therefore, our results indicate that S42909 might be a promising therapy to treat chronic ischemic skin defects. An appropriate dosage could improve the quality of life of many patients with this pathology.
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P7 - Placental structural alterations in pregnant women with varicose vein pathology

Mesa-Ciller, C.[1]; Álvarez-Rocha, MJ.[1]; Asúnsolo, A.[2]; Payá, P.[3]; Cifuentes, A.[1]; García-Honduvilla, N.[1]; Buján, J.[1]

[1]Department of Medicine and Medical Specialities
[2]Departement of Surgery, Medical and Social Sciences, Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain. Networking Biomedical Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.
[3]Servicio de Obstetricia y Ginecología. Hospital del Henares. Madrid, Spain.

Introduction: Placenta is an organ derived from both the mother and the fetus involved in transport, metabolic, protective and endocrine functions. Maternal blood enters at high pressure into the chorionic plate, and then slowly flows around the villous area when pressure lowers, allowing the exchange of substances, returning afterwards through veins. Changes in hemodynamics of maternal blood flow can seriously affect the growth of the placenta. The aim of this study was to evaluate the possible histological changes of the placenta on pregnant women with varicose pathology.
Methods: Human full-term placentae were collected after delivery and divided into two groups: control (n=15, women without varicose veins), and varicose (n=20, women with varicose pathology). Each group was subdivided into young (<35 years) and aged women (≥35 years). Histological sections were H-E stained, and light microscopy images (5 per sample, 100x) were analyzed to determine the number of villi and syncytial knots. Results were expressed as mean ± standard error of the mean and analyzed with Mann-Whitney U test.
Results: Placentae from women with varicose veins showed higher number and smaller size of villi than control group ($p<0.05$). Quantification of the syncytial knots revealed an increment in the varicose group when compared to control ($p<0.01$). Regarding the age, greater number of knots were also observed in young varicose vs. young control group ($p<0.01$). Fibrinoid deposits did not show significant differences between varicose and non-varicose group.
Conclusion: Observed differences between control and varicose groups suggest a relationship between CVI and the number of villi and syncytial knots. However, while this raise of villi seems to happen regardless of the age of the pregnant mother, the increment of syncytial knots could be mainly attributable to differences between young healthy and varicose populations.
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P8 - Exploring the effect of posture on the peripheral cutaneous microvascular response to the topical application of methyl nicotinate

Henrique Silva[1,2], Catarina Rosado[1], Hugo Ferreira[3], Joana Antunes[2] and L Monteiro Rodrigues[1,2]

[1]CBiOS – Universidade Lusófona's Research Center for Biosciences and Health Technologies (UDE), Campo Grande 376, 1749-024, Lisboa, Portugal
[2]Pharmacol. Sc Depart – Universidade de Lisboa, School of Pharmacy, Lisboa, Portugal
[3]Institute of Biophysics and Biomedical Engineering, Faculty of Sciences of the University of Lisbon

Cutaneous microcirculation reactivity is often studied with topical drug application, and the following response quantified with Laser Doppler Flowmetry (LDF). Nicotinates are vasodilator drugs often used for these purposes. The effects of age, gender, circadian rhythms and skin location on this vasodilation response have been studied before. However, the effect of posture still needs clarification. Our objective was to compare the vasodilation response elicited with the topical application of methyl nicotinate (MN). 14 young healthy volunteers (22.7 ± 2.8 years old) participated in this study, after giving their informed consent. MN was applied on the dorsum of a randomly chosen foot (a) while sitting and (b) while lying supine. The microcirculation response was evaluated with LDF (PF 5010 system, Perimed, Sweden) for 15 minutes after MN application. The first 5 minutes of each volunteer's LDF signal from the plateau phase were analyzed with: the wavelet transform, which gives its main frequency components (heart, respiration, myogenic, sympathetic and endothelial); the detrended fluctuation analysis (DFA), which translates correlations within the signal through the calculation of an alpha exponent; and the multiscale entropy analysis (MSE), which assesses the complexity of the signal. Statistical comparison between protocols was done with the Mann-Whitney test for independent samples. Results showed significantly lower respiratory and myogenic activities while lying supine as well as a significantly higher endothelial activity. The alpha exponent for the heart component was found to be significantly higher while lying supine, while for the remaining components no significant differences were found. Entropy levels for the myogenic and sympathetic components were found to be significantly higher while sitting. Our results suggest that these analysis tools are sensitive to detect differences regarding the blood flow regulatory mechanisms on different postures.

P9 - Nailfold Capillaroscopy Criteria for Distinction of Primary and Secondary Raynaud's Phenomenon

Marta C. Amaral[1,2], Filipe S. Paula[1,2], Joana Caetano[1],Isabel A. Ferreira[1,2], Susana Oliveira[1], J. Delgado Alves[1,2]

[1]Immune-Mediated Systemic Diseases Unit, Department of Medicine IV, Fernando Fonseca Hospital, Amadora, Portugal
[2]CEDOC - Chronic Diseases Research Centre, NOVA Medical School/Faculdade de Ciências Médicas, Universidade Nova de Lisboa

Raynaud's Phenomenon (RP) can be primary or secondary to immune-mediated diseases (ID). We had previously suggested nailfold videocapillaroscopy (NVC) parameters to distinguish primary from secondary RP: major capillary abnormalities, enlarged/giant capillaries (caps), number of caps/mm, type and velocity of circulation. Later on, microhemorrhages (MH) have been added.
Objectives: We aim to determine sensitivity, specificity and predictive values(PV) of our modified criteria.
Methods: Patients(pts) with RP were recruited and NVC was performed. They classified as secondary RP if they had at least 3 of: >5% major abnormal caps, enlarged/giant caps, <8 caps/mm, microhemorrhages, reduced red blood cell(RBC) velocity, intermittent flux with "sludge".
Results: 416pts, 368 women (89%), mean age 43yrs. 235(57%) had >5% major abnormal caps, 217(52%) had enlarged/giant caps, 179(43%) had <8 caps/mm, 117(28%) had MH, 233(56%) had reduced RBC velocity, 248(60%) had intermittent flux with "sludge".
137(33%) patients had non-ID associated RP, 89(21%) had systemic sclerosis, 140(34%) had another ID (SLE, RA, vasculitis), 50(12%) had an undifferentiated ID.
According to our criteria, 151(36%) pts classified as primary RP and 265(64%) as secondary RP, with sensitivity 73%, specificity 80%, positive PV 62%, negative PV 87%, P<0.0001.
Discussion: This methodology continues to be able to identify primary RP. The addition of microhemorrhages appears to increase its specificity.

P10 - Application of photoplethysmography to monitor heart rate

João Requicha, Clemente Rocha, Rui Assunção, Henrique Silva, Margarida Estudante, Luis Lobo, Luís Monteiro Rodrigues

CBIOS – Universidade Lusófona's Research Center for Biosciences and Health Technologies, U Lusófona, Av Campo Grande 376, 1749 024 Lisboa, Portugal

Photoplethysmography (PPG) is a noninvasive optical technique commonly used for pulse oximetry and blood pressure measurement. Such practical technology may offer other interesting possibilities for biometry. The present study explores an easy-to-use hardware platform for acquisition of biosignals and real-time wireless transmission by applying PPG to measure heart rate (HR) in vivo and compare it with a current HR monitor.
This study was developed in the U Lusofona Veterinary Faculty facilities, using one anesthetized adult mongrel dog (female 15kg). All experiments were carried out respecting the relevant EU and National rules on animal care. HR (in bpm) was monitored by a PPG sensor combined with the hardware platform Bitalino (Plux Biosignals, P) and compared with the VetCare monitor (Braun, FRG) which served as a positive control and manual register, in two experimental occasions (E1 and E2). Several anatomical locations (ear, tail and tongue) were tested and measured.
Mean heart rates obtained in E1 were 108.91+ 7.36 from manually register; 108.71+ 4.74 registered from the VetCare monitor and 105.86 + 7.90 from the Bitalino sensor. In the E2 , results were 105.87 + 5.30 from manual register, 107.64 + 6.94 from the VetCare monitor and 101.40 + 9.32 from Bitalino sensor.
Although preliminary, this approach suggests that the PPG technique can be used to monitor HR and evaluate the peripheral pulse synchronization with the heartbeat. Nevertheless, further developments are needed to establish its reliability, in particular in terms of repeatability and variability, in order to establish its real usefulness for these purposes. Additionally, it is also clear that the ear is the anatomical location providing better readings. This is an interesting alternative in veterinary practice where other locations (typically the foreleg and the tail) are frequently chosen despite the difficulties that hairy regions offer to sensor adaptation.

P11 - Different uptake of native versus aggregated LDL by aortic valve interstitial cells in normolipidemic-like in-vitro conditions

Antonella Bonetti[1], Vicenta Llorente-Cortés[2], Alberto Della Mora[1], Magali Contin[1], Franco Tubaro[3], Maurizio Marchini[1], Fulvia Ortolani[1]

[1]Department of Experimental and Clinical Medicine, University of Udine, 33100, Udine, Italy
[2]Centro de Investigación Cardiovascular CSIC-ICCC, Hospital de la Santa Creu i Sant Pau, 167 08025, Barcelona, España
[3]Department of Food Sciences, University of Udine, 33100, Udine, Italy

During atherosclerosis disease, macrophages and vascular smooth muscle cells internalize aggregated low density lipoprotein (agLDL) at much greater extent than native LDL (nLDL). Here, primary cultures of aortic valve interstitial cells (AVICs) were treated for 3 up to 21 days with 50 g/ml blood-derived nLDL or agLDL, alone or combined with pro-calcific culture media, to ascertain whether (i) agLDL are avidly taken up also by AVICs and (ii) treatment with LDL normolipidemic-like concentration can influence AVIC mineralization. Ultrastructurally, uptake by AVICs resulted to be restricted to agLDL, in accordance with chromatographic estimation of large amounts of intracytoplasmic esterified cholesterol and triglycerides after treatment with agLDL alone. Moreover, LDL were found to exert opposite effects on AVIC mineralization, with pro-calcific cell degeneration being mitigated after treatment with nLDL or exacerbated after treatment with agLDL. Consistently, spectrophotometrical estimations revealed calcium amounts to be decreased in the former cultures and increased in the latter cultures. The calcific process was found to depend on intracellular release of lipidic material and its layering at cell edges and cell debris, acting as major hydroxyapatite nucleator, in the same manner previously found for in-vivo experimental and pathological aortic valve mineralization. These preliminary data suggest that agLDL uptake by AVICs might contribute to lipid accumulation within aortic valves, besides affecting valve mineralization even at normolipidemic concentrations, strengthening the concept that valve stenosis is to be conceived as a valve "atherosclerotic lesion".

P12 - Testing tissue protective capacities of plant extracts

Patrícia Rijo[1,2], Marisa Nicolai[1], Ana Sofia Fernandes[1,2], Luís M. Rodrigues[1,3*]

[1]CBIOS – Universidade Lusófona Research Center for Biosciences & Health Technologies, Campo Grande 376, 1749-024 Lisboa, Portugal
[2]Research Institute for Medicines and Pharmaceutical Sciences (iMed.ULisboa)
[3]Dep Pharmacol Sciences, Universidade de Lisboa - Fac. Farmácia, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

Tissue damage occurring in chronic processes is known to involve common mechanisms that affect the extracellular matrix (ECM). As an example, regarding the human skin, UV radiation is recognized as a major aggressor, producing connective tissue alterations via the formation of lipid peroxides and other reactive oxygen species (ROS), and disturbing cell enzymatic defenses.
In recent years many materials, in particular from natural origin, have been studied in an attempt to disclose more effective molecules in tissue protection. Plant extracts have been reported as inhibitors of proteinases and as antioxidants, suggesting their roles in tissue remodeling in health and disease. Several micromethods and biomarkers have been developed with the purpose of assessing such properties.
In this work, we studied extracts of Plectranthus plants, as well as compounds isolated from these extracts, towards a potential application in tissue protection. In this regard, we describe the chemical composition of Plectranthus aqueous extracts (studied by HPLC-DAD), the antioxidant activity (using the DPPH method), the anti-tyrosinase properties and the acetylcholinesterase inhibition (assessed by the Ellman assay). We assessed the impact of the samples in the viability of human keratinocytes cultures. In addition, we showed that Parvifloron D, a diterpene isolated from Plectranthus ecklonii, might protect ECM from proteolytic degradation.
The interest of Plectranthus extracts as bioactive ingredients is related with their content in polyphenols (ex: rosmarinic acid). Polyphenols are among the antioxidants that are able to protect human skin against the harmful effects of ultraviolet irradiation, specifically against photo-aging. Our future investigations will focus on the assessment of anti-collagenase and anti-elastase properties of these plant extracts/compounds, in order to further explore their potential in tissue protection.