L.I.A.C
Latinorum Investigatorum
de Arteriis Colloquium
The Latin Society for Vascular Research

# XXVIIIth LIAC meeting on Vascular Research,

# Grenoble,

# October 3rd-6th, 2012

# ABSTRACT BOOK

UNIVERSITE JOSEPH FOURIER
SCIENCES.TECHNOLOGIE.SANTÉ GRENOBLE - ALPES

Instituts thématiques
Inserm
Institut national
de la santé et de la recherche médicale

Saint-Martin-d'Hères

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Coffee or tea will be available at 8h30 every morning, Thursday through Saturday.

Wednesday evening session located at the “Heure Bleue”, avenue Jean Vilar, St Martin d’Hères.

All other sessions located at the Faculty of Medicine and Pharmacy, Domaine de la Merci, avenue des maquis du Grésivaudan, La Tronche.

# Program

**Wednesday evening (OCT 3rd):**

- 16:00 – 18:00 : Registration

- 18:00 – 19:00 : **Opening ceremony (L'Heure Bleue – St Martin d'Hères) – Institutional welcome by Pr. Anne Milet -vice-president of Joseph Fourier University, in charge of the international relations, Pr. Christophe Ribuot, doyen of the Faculty of Pharmacy (Joseph Fourier University), Dr. René Proby - Mayor of Saint Martin d'Hères and David Queiros, 1st vice-mayor of St Martin d'Hères, and Pr. Michel Spina (University of Padova), president of the LIAC society.**

- 19:00 – 20:00 : Sciences and Philosophy: **Differences between animals and human beings - Bioethics (Nicolas Aumonier, Grenoble)**

- 20:00 : Wine and cheese contest (each group should bring a sample of wine and cheese from its region)

**Thursday morning (OCT 4th):** **SESSION I:** MOLECULAR AND SUPRAMOLECULAR STRUCTURE

Chair: Brigida Bochicchio and Smail Khelili

- 9:30-10:00 : **Antonietta Pepe (Potenza, Italy) : Elastin-derived peptides and the role of hydroxyproline**

- 10:00- 10:30 : **Brigida Bochicchio (Potenza, Italy) : From biopolymers to biomaterials: elastomeric protein-inspired polypeptides**

- 10:30 – 11:00 : Coffee break

- 11:00 – 11:30 : **Colette Lacabanne (Toulouse, France) : Broadband molecular dynamics in tropoelastin and its analogous fragments: influence of pathology on the physical structure**

- 11:30 – 12:00 : **Marie Joyeux-Faure (Grenoble, France): Atorvastatin protects against deleterious cardiovascular consequences induced by chronic intermittent hypoxia. (moved from session V)**

**12h30 : LUNCH**

**Thursday afternoon (OCT 4th):** **SESSION II:** BIOMATERIALS AND TISSUE ENGINEERING

Chair: Michel Spina and Natalio Garcia Honduvilla

- 14:00 – 14:30 : **Filippo Naso (Padova, Italy) : The relationship between the amount of alpha-Gal epitopes found in xenogenic bioprostheses and the threat of adverse clinical outcomes of current heart valve substitutes**

- 14:30 – 15:00 : **Catherine Picart (Grenoble, France) : Biomaterials and stem cell differentiation**

- 15:00 – 15:30 : **J Carlos Rodríguez-Cabello (Valladolid, Spain) : Tailored Design of Elastin-like Recombinamers for Biomedical and Biotecnological uses**

- 15:30 – 16:00 : **V. La Carrubba (Palermo, Italy) : Biodegradable Poly lactic-acid based scaffolds for soft tissue engineering and vascular tissue engineering**

- 16:00 – 16:20 : Coffee break

- 16:20 – 16:35 : **Michel Spina (Padova, Italy): Self-seeding heart valve design: Self-assembling peptide hydrogel as filler for decellularized pericardium**

- 16:35 – 16:50 : **Barbara Pérez-Köhler (Alcala de Henares, Spain): Are mesenchymal Wharton´s jelly cells a viable alternative to endothelial cells for its use in arterial substitution ?**

**17:00 – 18:00 : LIAC business meeting**

**Friday morning (OCT 5th): SESSION III:** SIGNALING AND CARDIOVASCULAR APPARATUS

Chair : Laurent Duca and Eric Esteve

- 9:00 – 9:30 : **Gustavo Egea (Barcelona, Spain) : Oxidative stress response in Marfan syndrome**

- 9:30 – 10:00 : **Smail Khelili (Jijel, Algeria) : Design, synthesis and pharmacological evaluation of new activators of ATP-dependent potassium channels**

- 10:00 – 10:30 : **Laurent Riou (Grenoble, France) : Assessment of mechanical stress in aortic atherosclerotic lesions from ApoE-/- mice. Comparison to human lesions**

- 10:30 – 11:00 : Coffee break

- 11:00 – 11:20 : **Alain-Pierre Gadeau (Pessac, France) : Sonic Hedgehog mediates mural cell recruitment to neovessels**

- 11:20 – 11:35 : **Mourad Bouhedja (Jijel, Algeria): Design, synthesis and ex vivo study of the vasorelaxant activity of new acyclic analogs structurally related to cromakalim and bearing a**

**arylsulfonylurea moiety**

- 11:35 – 11:50 : **Nadjib Kihal (Jijel, Algérie): Synthesis and evaluation of some benzothiadiazins derivatives on the contractile activity of rat aorta**

- 11:50 – 12:05 : **Zeinab Ghandour (Grenoble, France): Effect of microfibrils on calcium signaling and extracellular matrix synthesis in human endothelial cells (HUVEC). Role of the elastin receptor.**

- 12:05 – 12:20 : **Wassim Fhayli (Grenoble, France): Pharmacological validation of dill extract as a new anti-aging agent for the cardiovascular system**

**12:30 : LUNCH**

**Friday afternoon (OCT 5th): SESSION IV:** CARDIOVASCULAR PHYSIOPATHOLOGY AND MODELING

Chair : Fulvia Ortolani and Philippe Charpiot

- 14:15 – 14:45 : **Stéphanie Salmon (Reims, France) : Modeling of the hemodynamics**

- 14:45 – 15:15 : **Valdur Saks (Grenoble, France) : Energetic modeling in the cardiovascular system**

- 15:15 – 15:45 : **Antonella Bonetti (Udine, Italy) : Inorganic-phosphate-dependent autophagocytosis derangement and calcific events : Responsivity of aortic valve interstitial cells in cultures at different normophosphatemic-like conditions**

- 15:45 – 16:05 : Coffee break

- 16:05 – 16:35 : **Patrick Lévy (Grenoble, France) : Sleep apnea and cardiovascular pathologies (moved from session V)**

- 16:35 – 16:50 : **Laurent Duca (Reims, France) : Elastin Receptor Complex and elastin-derived peptides in atherosclerosis development (moved from session III)**

**18:30 : EXCURSION: VISIT OF OLD DOWNTOWN GRENOBLE**

**20:00 : GALA DINNER**

**Saturday morning (OCT 6th): SESSION V:** CLINICAL APPROACHES

Chair : Jacques Bonnet and Marilena Formato

- 9h30 – 10h00 : **Jose Delgado Alves (Lisboa, Portugal) : Plasma lipids and the humoral response in atherogenesis**

- 10h00 – 10h30 : **Alexis Broisat (Grenoble, France) : Anti mouse/human VCAM-1 nanobodies for SPECT imaging of atherosclerosis**

- 10:30 – 11h : Coffee break

- 11:00 – 11h30 : **Antonio Junior Lepedda (Sassari, Italy) : A proteomic approach to identify circulating and tissue biomarkers of atherosclerosis**

- **11:30-12:00 : Conclusion - Closure of the meeting**

- **12:00 - : SNACKS**

Wednesday evening, October 3rd

**Sciences and philosophy conference**

# Différences Homme - animal

Nicolas Aumonier, Université Joseph Fourier, Grenoble, France

Y a-t-il vraiment une différence entre l'être humain et l'animal et, si tel est le cas, comment pouvons-nous la caractériser ? La biologie tend plutôt à les confondre, la comparaison de nos comportements et de nos mœurs avec les leur, plutôt à nous distinguer d'eux. Est-ce parce qu'il existe une différence réelle entre l'un et l'autre, ou parce que cette distinction n'est que symbolique ? Ces questions ne touchent pas seulement la possibilité ou non d'expérimenter sur les animaux, mais aussi la manière d'être un être humain, cherchant à se comporter humainement.

**Session I**

# Elastin-derived peptides : comprehending the role of hydroxyproline in the mature elastin

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Tropoelastin undergoes several post-translational modification before maturation into elastin. The hydroxylation of some proline residues by prolyl-4-hydroxylase to form (2S,4R)-4-hydroxyproline (Hyp) is one of the post-translational modification of elastin. In order to define the role played by Hyp in the function and self-assembly of the mature protein, elastin model peptides containing proline and analogues, Hyp and (2S,4R)-4-methoxyproline (Mop), were analyzed. Mop amino acid was introduced in order to distinguish between stereoelectronic effect (present in Hyp as well as in Mop) and the role played by the H-bonds (present only in Hyp).

Elastin peptides were analyzed by Circular Dichroism, NMR, and FTIR spectroscopies. The self-assembling of the peptides was studied by Turbidimetry assay, Atomic Force and Transmission Electron Microscopies. At molecular level, the conformational studies show that the presence of proline analogues, Hyp and Mop, instead of proline present in the elastin model peptides, did not significantly change the secondary structures populating the conformational space. As a matter of fact, PPII, unordered conformations and beta turns are still present. On the contrary, at supramolecular level the self-assembling properties instead are very different. The presence of Hyp reduces the tendency to coacervate and alters the morphology of the aggregates. The biological significance of these findings will be discussed.

**Session I**

**From biopolymers to biomaterials: elastomeric protein-inspired polypeptides**.

Bochicchio B., Bracalello A., Pepe A., Crudele M.A.
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Regenerative medicine and tissue engineering are new approaches to the treatment of severe cardiovascular diseases. In particular, products obtained by tissue engineering are emerging as valid alternative to bypass grafting with autologous veins or arteries not always available. As a matter of fact, tissue engineering approaches have been applied to use scaffolds coated with biocompatible polymers able to recreate the two main cellular layers of a normal artery and mimic the native vessel behavior: the intima, consisting of a monolayer of endothelial cells (ECs) that confers a thrombo-resistant surface, and the media, consisting of circumferentially aligned smooth muscle cells (SMCs). In this context, the use of protein-based biopolymers, able to mimic the properties of extracellular matrix, as coating material, as well as scaffold, is promising. We propose some chimeric polypeptides composed of resilin-, elastin- and collagen proteins as biomaterials in tissue engineering. The chimeric polypeptides, obtained by DNA recombinant technologies, were studied at molecular and supramolecular level. The mechanical as well as the biological properties were also investigated [1].

[1] A. Bracalello,Valentina Santopietro, Massimo Vassalli, Giovanni Marletta, Rosanna Del Gaudio, Brigida Bochicchio, and Antonietta Pepe. Design and Production of a Chimeric Resilin-, Elastin-, and Collagen-Like Engineered Polypeptide. Biomacromolecules 2011, 12, 2957–2965.

**Session I**

## Broadband molecular dynamics in tropoelastin and its analogous fragments: influence of pathology on the physical structure

Samouillan V.[1], Dandurand J.[1], Delaunay F.[1], Lacabanne C.[1], Bochicchio B.[2], Pepe A.[2], Nasarre L.[3], Badimon L.[3] and Llorente-Cortes V.[3]
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Various static techniques are well suited to evaluate the different structural levels of proteins both in the hydrated and dehydrated states, bringing to the fore structure/function relationships. The experimental analysis of molecular dynamics gives complementary information: the combination of calorimetry and broad band dielectric spectroscopy is worth being adapted to the analysis of polypeptides and proteins. In this talk we chose to explore the ability of these low frequency techniques to access the molecular dynamics of synthetic fragments of human tropoelastin (reductionist approach) with distinct conformations and architecture and human tropoelastin synthesized by smooth muscle cells in different conditions.

The first topic is devoted to the specific organization of a synthetic polypeptide (S4, derived from the exon 30 of human tropoelastin) released during the proteolysis of human tropoelastin by MMP12. The conformational and microscopy studies revealed that S4 polypeptide was able to form amyloid-like fibers under certain conditions. Furthermore, we chose to compare the low frequency chain dynamics of S4 and S4 fibers in order to extract the influence of the architecture on the molecular mobility of these systems. Differential Scanning Calorimetry (DSC) revealed the different thermal transitions such as glass transition and denaturation; the occurrence of a melting peak in S4 fibers thermograms was associated with the highly ordered structure (such as the cross-β spine structure evidenced in amyloid fibers). Dynamic Dielectric Spectroscopy (DDS) was used to scan the localized and delocalized motions, and a clear discrepancy was evidenced between S4 and S4 fibers at the level of the delocalized mobility. Thermally Stimulated Currents (TSC) completed the dielectric study giving information on the cooperativity of involved motions.

In the second topic, we compared the physical structure of two synthetic polypeptide sequences derived from exon 50 in order to get insight into the role of proline hydroxylation in native elastin; as a matter of fact this post-translational modification, which is well understood in the case of collagen, is still matter of debate in the case of elastin. In this case DSC clearly evidenced the importance of hydrophobic interactions with the formation of clathrate structures depending on the hydroxylation of proline.

In the last topic, we showed how aggregated LDL (agLDL), one of the main LDL modifications in the arterial intima that contributes to massive intracellular cholesteryl ester (CE) accumulation in human vascular smooth muscle cells (VSMC) could act on the physical structure and the molecular mobility of tropoelastin. Thermal and dielectric analyses evidenced that tropoelastin produced by agLDL-VSMC possessed decreased glass transition temperatures and distinct chain dynamics that besides a loss of thermal stability evidence strong changes in molecular mobility. It was shown how VSMC-lipid loading determines alterations in the mechanical properties of the vascular wall and plays a crucial role in elastin loss during atherosclerosis.

## Session II

### The relationship between the amount of alpha-Gal epitopes found in xenogenic bioprostheses and the threat of adverse clinical outcomes of current heart valve substitutes

Filippo Naso.
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Xenogeneic tissues are currently employed in clinical practice to create biological substitutes and in the repair of various damaged tissues, even if the presence of superficial epitopes as αGal are capable to trigger hyperacute and acute vascular rejection phenomena. Currently, neither glutaraldehyde fixation nor decellularization procedures ensure a definitive solution because of the persistence of reactive xenoantigen residues. In 2010 was developed the first assay able to quantify the αGal antigen concentration in a whole connective tissue, before and after detergent-based cell removal procedures, further up-graded in 2012 in order to extent the determination also in glutaraldehyde fixed tissues.

In the past, the lack of a reliable test providing quantitative information about the content of these epitopes in a tissue engineered scaffold before its clinical use, led to disappointing and disastrous results. In the 2003 the Synergraft experience caused the death of four children, in late 2007 the use of a bioprosthetic valve named Matrix P Plus, not tested for αGal content, resulted in inflammatory infiltration and onset of severe fibrogenic pseudointimal reaction in 38% of the operated patients. Incomplete removal of the αGal epitopes from xenogenic tissues was likely responsible for the rapid failure of bovine decellularised ureteric grafts as well as the degeneration of decellularised bovine or porcine cartilage and ligament. Tissue patches of xenogeneic origin used for pericardial closure, odontological purposes, eye and nerve surgery and ulcer repairs are commercially available even if in none of these the presence of residual xenogeneic antigens has been ever investigated. Finally the heart valves substitutes currently implanted in humans maintain a non-negligible presence of αGal epitopes able to significantly increase the circulating anti-αGal human antibodies from just 10 days after implantation. Its quantification might provide indications of biocompatibility relevant for the selection of bioprosthetic devices and an increase in the confidence of the patient, becoming a major quality control tool in the production and redirection of future investigation in the quest for αGal-free long lasting biological substitutes.

**Session II**

## Engineered biomaterial coatings : applications to musculo-skeletal tissue engineering

**Catherine Picart,**

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Beside events triggered by chemical ligands, mechanical properties of model and natural gels have recently been demonstrated to play an important role on various cellular processes such as adhesion, proliferation and differentiation. Bioactive signals such as growth factors are also important to dictate cell fate. Only few model materials exist that allow a systematic variation of their stiffness and/or bioactivity. We have developed self-assembled films based on extra-cellular matrix polysaccharides and polypeptides that can be coated on different types of biomaterials surfaces. These nanocoatings can deliver locally osteo-inductive proteins in a very efficient manner. The differentiation of muscle precursors in myotubes or in osteoblasts can be triggered by biochemical and mechanical properties of the films. These coatings present very interesting features for musculo--skeletal tissue engineering. In addition, they can also be used to investigate synergistic interactions between growth factor receptors and cell adhesion receptors.

**Session II**

**Tailored Design of Elastin-like Recombinamers for Biomedical and Biotecnological uses.**

Rodríguez-Cabello JC.
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**Introduction.**

Recombinamers are recombinant protein-based polymers. They are produced as recombinant proteins by genetically engineered microorganisms by the use of a synthetic DNA. One of the most successful family of recombinamers is the elastrin-like recombinamers (ELRs). Functional ELRs exhibit a reversible, LCST-like, phase transition in response to changes in temperature.

**ELR-based Biosurface engineering: Cell Harvesting.**

ELRs exhibit some advantages that make them excellent candidates for the development of responsive surfaces. Ozturk et al. have prepared micropatterned pNIPAM films as thermo-responsive cell carriers chemically modified by ELR adsorption containing RGD amino acid sequence to promote cell adhesion. They have studied the thermal responsiveness to apply mechanical stress on cells under in vitro conditions to induce bone formation5. However one more impressive use of ELRs is the design of smart surfaces that shows temporal control of cell-adhesion. To do that, a precise molecular design have been made such the LCTS behavior of ELRs can be used to promote a change on surface properties from cell adherent to cell antifouling. The transition from adherence to non-adherence is driven by a decrease in the temperature (from 37ºC to 15ºC), which cause cell detachment of the cell grown on that surface.

**ELR systems for tissue engineering**

ELRs of the type: $[(VPGIG)_2$-VPGKG-$(VPGIG)_2$- (EEIQIGHIPREDVDYHLPY)-$(VPGIG)_2$ -VPGKG-$(VPGIG)_2$-$(VGVAPG)_3]_n$ (n = 10; MW = 80925 Da) have been produced and designer for scaffold construction. The recombinamer contains the (REDV) peptide sequence which is specifically recognized by a few cell lines, specially by endothelial cells. The recombinamer includes a protease sensitive block, $(VGVAPG)_3$. This sequence was introduced to drive enzymatic hydrolysis of the synthetic scaffold by the same physiological pathways as natural elastin during ECM remodeling. Data of the performance of this, and some other versions of this recombinamer, will be shown for different tissue engineering applications.

## Session II

# Biodegradable Poly lactic-acid based scaffolds for soft tissue engineering and vascular tissue engineering

Vincenzo La Carrubba DICAM, Università di Palermo, Italy. Email: vincenzo.lacarrubba@unipa.it

### Introduction

Tissue engineering (TE) is an emerging multidisciplinary field involving biology, medicine, and engineering.

The most flexible, tuneable and promising methods for the preparation of 3D interconnected polymeric porous structures for tissue engineering (scaffolds) are based on liquid-liquid phase separation of polymer solutions, often called TIPS or DIPS (Thermally o Diffusion Induced Phase Separation, respectively). In TIPS, a stable polymeric ternary solution is brought below its metastability/instability point (spinodal/binodal curve) by quench: a foam-like structure is formed by nucleation and 3-D growth of the polymer lean phase, which, after solvent removal by rinsing and drying, will constitute the voids of the as-generated "open-pore" architecture. Within the field of TE scaffolds, a specific research topic concerns the so-called vascular tissue engineering, i.e. the idea of making tubular constructs in an attempt to develop a functional small-diameter arterial replacement.

In the following, some examples of morphologies of PLLA scaffolds for soft tissue engineering and vascular tissue engineering are reported, with some in-vivo testing showing the potential of the method proposed.

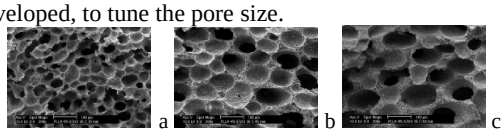
### Experimental methods

An homogeneous ternary solution composed by PLLA dioxane and water was prepared, with a constant dioxane (solvent) to water (antisolvent) weight ratio. The solution was hot poured into a sample holder. The temperature was then suddenly lowered to a value within the unstable region for a well-defined time interval, by pool immersion of the sample holder into a thermostatic water bath. Then a quench by pool immersion in an ethyl alcohol bath at a temperature of -20 °C was performed in order to freeze the as-obtained structure.

Scaffolds for vascular tissue engineering were obtained performing a DIPS process around a nylon fibre. The fibre was first immersed in a PLLA/dioxane solution at a fixed temperature. Then the fibre was slowly drawn at a constant rate from the solution and put in a second bath (DIPS bath), containing distilled water or dioxane/water at different ratios, at the same temperature, for a well-defined time interval. The fibre is first covered by a layer of polymeric solution; the immersion in the coagulation bath generates a microporous structure due to the diffusion of solvents.

### Results and discusssion

Fig.1 and 2 show how it is possible, via the TIPS protocol developed, to tune the pore size.



a b c

Fig. 1. PLLA scaffolds at 35 ° and 15 (a), 45 (b) and 60 min (c).

In vitro tests show that after 30 days of culture, the number of the cells into the scaffold was significant (fig. 3). Cells colonized the scaffold and a 3D structure was achieved. This is also confirmed by the optical images.

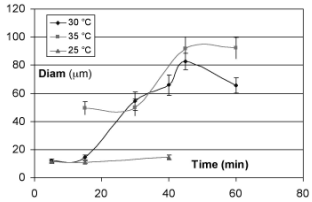
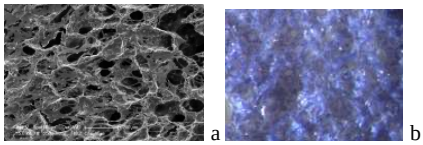



Fig. 2. Pore size of PLLA scaffolds vs. time and temperature



a b

Fig. 3. SEM (a) and optical (b) images of a scaffold after 30 days of fibroblast culture.

Fig. 4 shows a scaffold prepared via DIPS. Fig. 5 illustrates a laser confocal image of a scaffold with EC. The part coloured in grey are the nuclei of the cells, while the areas in white indicate the actin. After 30 days the internal lumen of the scaffold is covered by EC, organized into a real vessel structure; with stable cell-cell interactions; spidle membrane protrusion, characteristic of mesenchymal endothelial phenotype, were not observed.

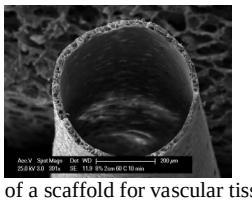
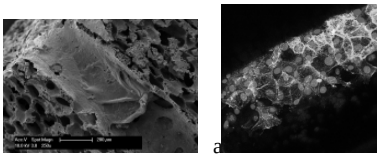


Fig. 4. SEM image of a scaffold for vascular tissue engineering



a b

Fig. 5. Scaffold after 30 days of culture SEM (a), confocal (b).

### References

1. Li S. , La Carrubba V., Piccarolo S., Sannino D., Brucato V., Pol Int, 53, (2004), 2079-2085
2. F. Carfì Pavia, V. La Carrubba, V. Brucato, S. Piccarolo J of Biom Mat Res A, 459-466 (2008)
3. F Carfì Pavia, G. Turturici, F. Geraci, V. Brucato, V. La Carrubba, C. Luparello, G. Sconzo, J. Biosci. 34 873–879 (2009)
4. F. Carfì Pavia, V. La Carrubba, V. Brucato, Polymer Bulletin (2012), in press
5. F. Carfì Pavia, V. La Carrubba, V. Brucato, G. Ghersi, Polymer Eng Science (2012), in press

**Session II**

## Self-seeding heart valve design: Self-assembling peptide hydrogel as filler for decellularized pericardium

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Heart valve biological prostheses generally have good hemodynamic characteristics and avoid long-term pharmacological therapies. However, the treatment with glutaraldehyde carried out in order to shield xenogenic epitopes to prevent rejection cannot avoid and/or leads to tissue deterioration after implantation with consequent necessity of new surgery after 10-15 years. Furthermore, all commercially available valve substitutes are nonviable and consequently their utility in surgery for children is limited. The goal of this investigation is to engineer self-seeding heart valves that allowing cell repopulation, could mature quickly in vivo and have a shorter preparation time. Self-assembling peptides (SAP) hydrogels could be used as filler to add biocompatible properties to decellularized scaffolds not treated with glutaraldehyde.

In the present study, we are demonstrating that self-assembling peptides can be used as filler to designing a new class of self-seeding easy-to-prepare heart valve substitutes. In this project, the decellularized pericardium is the 3D scaffolding element whereas the SAP hydrogel creates a fluffy nanofibrous environment able to call and house cells inside the natural decellularized scaffold. The capacity of SAP to penetrate and to remain inside the structure obtained simply by pre-incubation of decellularized pericardium in a peptide solution offers a very novel and smart approach. In addition, the already demonstrated capacity of SAP peptide to increase cell adhesion and growth, to deliver drug or proteins allowing their modulated release and the possibility to covalently decorate SAP scaffold with bioactive molecule induce optimistic forecasts in the prosecution of our project with *in vitro* and *in vivo* biological assays.

**Session II**

**Are mesenchymal Wharton´s jelly cells a viable alternative to endothelial cells for its use in arterial substitution?**

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**Introduction:** Vascular pathologies are one of the major morbidity and mortality reasons of the human being, which entails the need of having an adequate vascular substitute. The proper combination of scaffold, cell seeding and biological/biochemical signals can reduce the risk of graft failure while cooperating in the healing process activated after the graft implant, known as intimal hyperplasia.

**Material and Methods:** Vascular constructs were designed using fibronectin-precoated ePTFE grafts (4 cm length, 4 mm inner diameter, 30 μm internodal space) and different cell populations: endothelial cells (HUVEC), Wharton´s jelly cells (WJC) and Wharton´s jelly cells differentiated towards endothelial lineage (Dif-WJC). The study groups were established as follows: ePTFE control (without cell seeding), ePTFE+HUVEC, ePTFE+WJC and ePTFE+Dif-WJC (n=4 each). Constructs were grafted in both left and right common femoral artery (n=16) of Beagle dogs (n=8). After 60 days of study, constructs were collected. Morphometric, histological (conventional stainings), and immunohistological analyses (anti-activated macrophages) were set up in order to evaluate the intimal hyperplasia process.

**Results:** After 60 days of study, control group showed the highest occlusion rate (75%) and the thickest neointimal layer. Constructs seeded with HUVEC showed the lowest occlusion rate (25%) and a significantly thinner neointima compared with the control group. Both groups seeded with WJC and Dif-WJC showed lower occlusion rates than the control group (55% and 50%, respectively), but not as good as the HUVEC group. There was a gradual increase of the neointimal layer thickness ranging from the proximal to the distal anastomosis in all groups except ePTFE+Dif-WJC, which showed a roughly homogeneous thickness. All the experimental groups showed: moderate cell infiltration inside the ePTFE internodal spaces; low macrophagic reaction (except in the perianastomotic regions, where the presence of active macrophages is increased) and formation of a thick neoadventitial layer.

**Conclusion:** Differentiated cells from Wharton´s jelly stimulate the development of a thinner and more stable neointimal layer than the other cell populations, which could cooperate in the reduction of the restenotic processes triggered inside the vascular graft.

**Session III**

## OXIDATIVE STRESS RESPONSE IN MARFAN SYNDROME

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Marfan syndrome (MFS) is an autosomal dominant rare disease caused by mutations in the fibrillin-1 gene (FBN1), encoding fibrillin1, a major adhesive protein of the extracellular matrix (ECM) forming microfibrils. The syndrome carriers an increased risk of aortic dilatation (aneurysm), dissection and rupture. The syndrome also affects mitral valve, eyes, skeleton, skin, lungs and neurological system. Aneurysm and dissection of aorta are related to a progressive degradation of the ECM (collagens and elastin) of the arterial wall (tunica media and adventitia). In the physiopathology of MFS, matrix metalloproteases and activity of TGF-β (a growth factor involved in matrix and antiprotease secretion) are directly involved. Thus, from the cell and molecular point of view, MFS results from dysfunctions in the crosstalk between the intracellular mediators of signaling and the ECM in the cardiovascular system. NADPH proteins (Noxes) contribute to the production of reactive oxygen species (ROS), which, among other functions, participate in the maintenance of the differentiation state of VSMCs. ROS are particularly produced by cells in the vascular wall and it is known that TGF-beta controls the expression and enzymatic activity of Noxes in some cells. Here we examined in primary cultures of aortic VSMC derived from patients with the MFS the contribution of Noxes in the etiology of this disease. We postulated that as a consequence of the permanent activation of TGF-β occurring in MFS, VSMCs should show a differential gene expression of at least some family members. Thus, we have carried out RT-PCR of Nox genes (Nox 1, 2, 3, 4 and 5) and we observed that in Marfan VSMCs there is a significant decrease in Nox2 but an increase in Nox4 mRNA levels. Moreover, these changes are TGF-β dependent since the addition of a specific inhibitor of the TGF-β receptor I (LY364947) abolished these alterations. Interestingly, all Marfan cell lines also showed a robust actin cytoskeleton organization, which correlated well with the observed increase in Nox4 expression levels, since Nox4 is an activator of RhoA. In conclusion, our results suggest the involvement of NADPH oxidases in the cardiovascular alterations associated with the promiscuous TGF-β stimulation occurring in Marfan syndrome.

**Session III**

## Design, synthesis and pharmacological evaluation of new activators of ATP-dependent potassium channels

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The ATP-dependent potassium channels (KATP channels) are involved in many physiological processes, namely the control of insulin secretion by pancreatic β cells, the contractile activity of some types of muscles (vascular smooth muscle and non-vascular) and the protection of several types of cells (heart, kidney and nervous cells) under conditions of metabolic stress. Therefore, these channels have been for decades the target of numerous research to develop molecules able to inhibit or activate them, in order to be used as drugs in the treatment of various diseases such as diabetes, hypertension, asthma .... etc.. Indeed, sulfonylureas, inhibitors of KATP channels, have been used for decades in the treatment of non-insulin-dependent (type II), whereas pinacidil, diazoxide and nicorandil are antihypertensive drugs. The latter is also used against angina. Other molecules, such as cromakalim, initially very promising, failed to pass the clinical phase IV because of its toxicity (Figure 1). However, scientific work in search of new modulators of KATP channels continues.

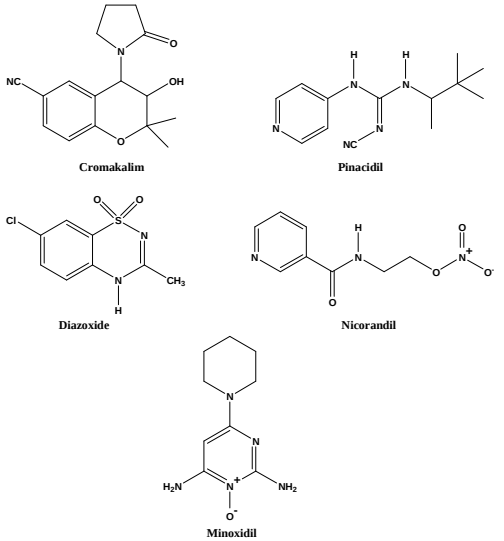


Figure 1. Exemples of ATP-dependent potassium channels activators.

The KATP channel is a complex made up of four octameric units Kir6.x (inwardly rectifying family of potassium channels), forming the channel pore, and four subunits SURx (sulfonylurea receptor). The nature and isoforms Kir6.x and SURx determine the effectiveness of action and tissue selectivity of various chemical agents against these channels. Indeed, cromakalim is selective of vascular smooth muscle cells while diazoxide is active on both vascular smooth muscle and pancreatic β cells.
It is reported that activators and inhibitors of KATP channels bind to the receptor at close sites. In order to develop new molecules activating KATP channels, we adopted a strategy that consists in the combination, on the same chemical skeleton, structural elements belonging to both an activator and an inhibitor of KATP channels, or structural elements of two activators. The process can be a simplification of the structure or can goes in the direction of complexity. We chose as a model of activators diazoxide and cromakalim, while sulfonylurea will be the model of inhibitor. The synthesized molecules were

evaluated on three types of cells: pancreatic b cells, vascular smooth muscle cells and uterine cells. The obtained results will discussed with more details.

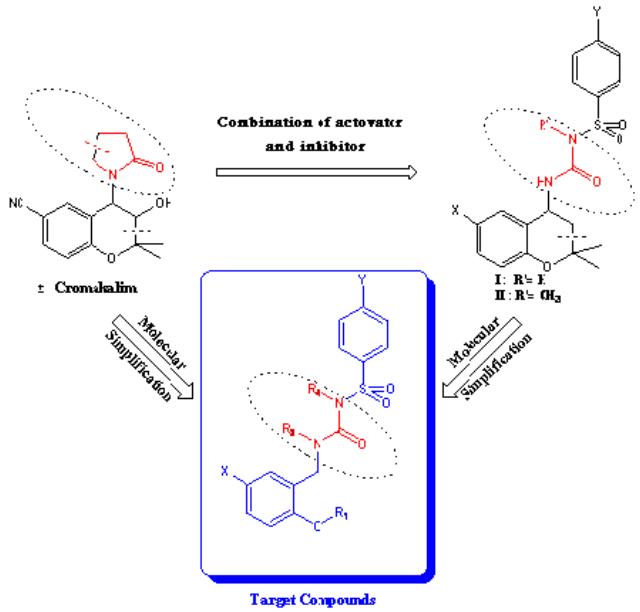


Figure 2. Combination of structural elements an activator (cromakalim) and an inhibitor (sulfonylurea).

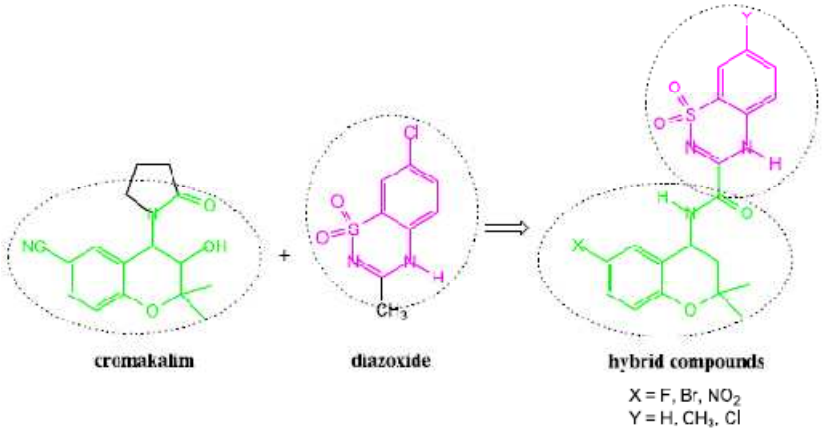


Figure 3. Combination of structural elements two activators : cromakalim and diazoxide.

**Session III**

## Assessment of Mechanical Stress in Aortic Atherosclerotic Lesions from ApoE-/- Mice – Comparison to Human Lesions

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Despite biological processes similar to those observed in humans, atherosclerotic plaque rupture does not occur in apoE$^{-/-}$ mice. Since mechanical stress impacts on human plaque rupture, our objectives were to quantify regional mechanical stresses in apoE$^{-/-}$ mice atherosclerotic lesions and to correlate stress distribution with biological parameters of atherosclerotic plaque development.

Aortic rings were obtained from 7- to 30 weeks-old apoE$^{-/-}$ and control mice and cut radially to release residual stresses and strains (RSS). Immunohistology and biomechanical modeling were performed to determine stress distribution from plaque composition. An increase in RSS was observed in apoE$^{-/-}$ animals, which was correlated with media & adventitia thickness and elastic lamella tortuosity ($P<0.05$) but not with neointimal thickness. Maximal stress amplitude was decreased by 14-, 2.9-, and 3.6-fold in plaque shoulder, center and back areas, respectively, when RSS were considered. Maximum stress values were observed in the normal arterial wall rather than the atherosclerotic lesions. Stress distribution was not correlated to macrophage infiltration in lesions.

In conclusion, low mechanical stress amplitude in apoE$^{-/-}$ mice lesions in comparison to that previously reported in humans probably accounts for the lack of plaque rupture in this widely used model.

**Session III**

## Sonic Hedgehog mediates mural cell recruitment to neovessels

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**Background:** Recruitment of mural cells, i.e. pericytes and smooth muscle cells (SMC), is an essential step to insure newly formed vessel maturation. Sonic hedgehog (Shh) has been suggested to promote the formation of larger and more muscularized vessels, but the underlying mechanisms of this process have not yet been elucidated. Platelet-Derived Growth Factor BB (PDGF BB) is known to be involved in this process and we hypothesized that the Hedgehog pathway may work in conjunction with PDGF BB to promote mural cell migration and recruitment into neovessels.

**Methods and results:** We used the mouse corneal angiogenesis model to investigate our hypothesis and found that PDGF BB and Shh were expressed by endothelial cells and mural cells of growing blood vessels respectively and that Shh is a target of PDGF BB. Moreover, we demonstrated that inhibition of either PDGF BB or Shh signaling reduced $NG2^+$ mural cell recruitment into neovessels and subsequently reduced the lifespan of neo-vessels. We then further characterized the role of Shh *in vitro* and showed that Shh or Smoothened (Smo) inhibition reduces PDGF BB-induced SMC migration Moreover, we found that PDGF BB-induced SMC migration, involves Shh-dependent activation of PI3Kγ, ERK1/2 and Gli-dependent transcription.

**Conclusions:** This study demonstrates, for the first time, that Shh is a key mediator of PDGF BB-induced mural cell migration and recruitment into neo-vessels and elucidates the molecular signaling pathway involved in this process.

**Session III**

## DESIGN, SYNTHESIS AND EX VIVO VASORELAXANT ACTIVITY OF NEW ACYCLIC ANALOGUES, STRUCTURALLY RELATED TO CROMAKALIM, BEARING A ARYLSULFONYLUREA MOIETIES

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In the present work, ring-opened analogues **(A, B)** of dihydrobenzopyran potassium channel openers (PCOs) **(I, II),** were prepared and evaluated on rat aorta rings (myorelaxant effect). These derivatives are characterized by the presence of a sulfonylurea function. The prototype compounds of general formula **(II)** exhibited a marked myorelaxant activity on the vascular and uterine smooth muscle tissues [1,2].

Series **A** and **B**, structurally related to cromakalim, a potent activator of ATP-dependent potassium channels, were synthesized and purified. Confirmation of chemical structures was performed by usual spectroscopic methods of analysis (MS, IR, $^{1}$H NMR) and elemental analyses. And their vasorelaxant activities have been evaluated on isolated rat thoracic aorta rings precontracted with 30 mM KCl, removed from adult fed Wistar rats (200–400 g) [3]. The pharmacological results indicated that the myorelaxant activity of the non N-methylated analogues (**A)** was less pronounced than those of N-methylated compounds (**B)**.

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**Session III**

## Synthesis and evaluation of some benzothiadiazins derivatives on contractile activity of rat aorta

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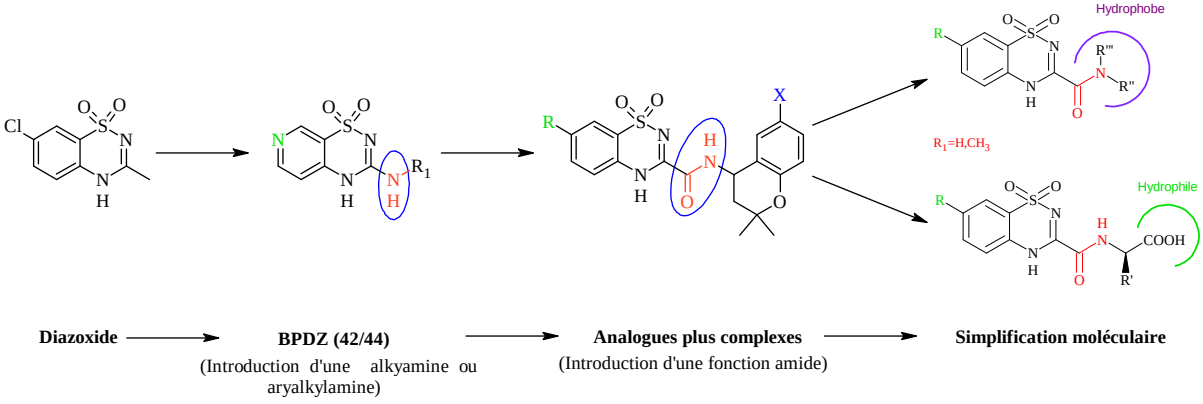
ATP-sensitive potassium channels ($K_{ATP}$) play a crucial role in the control of membrane potential. The activation of these channels, via the release of potassium ions from the cell, leads to hyperpolarization of the plasma membrane and therefore regulates many physiological processes [1, 2, 3].

Based on previous results [2, 4] and in order to develop original diazoxide analogues, with more potent and more tissue-selective properties, and improved physico-chemical properties, especially their solubility, we synthesized two series of benzothiadiazin-1,1-dioxides substituted by α-amino acid, aliphatic or aromatic amine, linked to the 3-position of the heterocycle by an amide function (see figure).




In order to evaluate the pharmacological profile of our analogues, a measure of the residual isometric contraction of rat aorta was performed [3]. Dose-effect curves of these compounds were established and discussed.

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**Session III**

## Effect of microfibrils on calcium signaling and extracellular matrix synthesis in human endothelial cells (HUVEC): Role of the elastin receptor

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Microfibrils (MF) are protein complexes with a diameter of 10-12nm, composed of more than 17 molecules, and are one of the two essential constituents of elastic fibers. Fibrillin-1, a protein with a high molecular weight, is the principal component of MF and plays an essential role in elastic fiber formation. MF has been demonstrated to be able to induce an increase in intracellular calcium level in endothelial cells HUVECs. Our experiments show that this calcium elevation level is essentially mediated by the release of intracellular calcium stores from the endoplasmic reticulum (ER), reinforced by the influx due to the activation of membrane calcium channels. MF induced intracellular signaling pathways across MEK1, Src and phospholipase C which activated $IP_3$ receptors in ER membrane involving calcium release in the cell. These MF signal-related intracellular events were mediated by integrins and, mainly, the elastin receptor. MF application to rat aorta rings induced an endothelial production of nitric oxide (NO) which produced a relaxation of vascular smooth muscle cells and a strong vasodilation. In addition, it has been observed that MF likely contribute to the regulation of the vascular structure and elasticity: 48 hours after MF treatment of HUVEC, increases in elastin synthesis (+110%), fibrillin-1 (+20%) and collagens (+50%) were observed. All these results support the hypothesis that microfibrils positively control the vascular elasticity, hypothesis that can explain the appearance of vascular aneurysm in Marfan syndrome, due to fibrillin-1 deficiency and subsequent abnormal MF formation.

**Session III**

**Pharmacological validation of dill extract as a new anti-aging agent for the cardiovascular system**

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Elastic fibres are extracellular matrix components made of elastin (90%) and microfibrils (10%). Elastin is synthesized only in early life and confers to the large elastic arteries the property of elasticity, allowing storage of energy and stroke volume during the systole, then release of energy and blood volume during the diastole. This phenomenon, known as the windkessel effect, helps to decrease the load on the heart, minimize the systolic flow and maximize the diastolic flow in the arteries. With aging, elastin is progressively degraded, leading to arterial stiffening, and dysfunction. However, an aqueous dill extract (DE) has been shown *in vitro* to stimulate elastin expression (elastogenesis) in skin. In this study, we investigated the role of this extract (DE 5% and DE 10% v/v) on the arterial structure and function *in vivo* using 24-month-old aged mice (C57Bl6/J). Mice treated for 3 months with 5% DE had a lower mean (72±2.03 mmHg vs 78±2.49 mmHg) and systolic blood pressure (80±1.5 mmHg vs 90.8±0.88 mmHg), when compared to untreated mice, respectively. Also, 5% DE induced a significant decrease in the total heart- (0.44± 0.04 vs 0.57±0.01), left ventricle- (0.25±0.02 vs 0.31±0.007), septum- (0.08±0.007 vs 0.12±0.008) and right ventricle- (0.09±0.008 vs 0.12±0.006) weight to body weight ratios, compared to those of untreated mice. Treatment with 10% DE had no effect on both mean arterial blood pressure and heart weight. We also investigated the mechanical properties of cannulated abdominal aorta by pressure arteriography. The abdominal aorta underwent a DE-induced increase in diameter and wall thickness. Moreover, aortas treated with DE presented a significant increase in distensibility at low pressure (0-25 mmHg) compared to their counterparts. At the same strain ratios, abdominal aortas treated by the extract had decreased circumferential stress values due to the increased wall thickness, compared to their untreated counterparts. Vessel stiffness (Einc) was found slightly lower in animals treated by DE compared to non-treated animals. Histological studies were carried out by microscopic observation of Weigert- (for elastic fiber) and Eosin/Hematoxylin-stained paraffin-embedded vessels cross-sections. There was no obvious structural difference between treated and non-treated animals. In conclusion, our results suggest that DE presents an interesting potential for arterial function improvement in an anti-aging perspective, even when treating already aged animals.

**Session III**

**Elastin Receptor Complex and elastin-derived peptides in atherosclerosis development**

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**Introduction and Objectives :** The role of inflammation in atherosclerosis has been extensively studied and many works describe elastin peptides (EP) as a regulator of major biological mechanisms that could impact on atherosclerotic plaque progression. Interestingly, PI3Kγ has been identified as a key protein of the elastin receptor complex (ERC) signaling induced by elastin peptides (EP) and its absence in the hematopoietic lineage prevented atherosclerosis initiation and development. After summarizing the litterature in the domain, last data depicting the role of EP in atherosclerosis through PI3Kγ will be presented.

**Materials and Methods :** experiments were conducted using ApoE$^{-/-}$ and LDLr$^{-/-}$ mice. Involvement of PI3Kγ in the EP-induced atherosclerosis was studied using chimeric LDLr$^{-/-}$ mice devoid of PI3Kγ in the immune system. *In vitro* influence of EPs and ERC in monocytes migration and ROS production was also evaluated.

**Results :** EP injection to ApoE$^{-/-}$ mice accelerates atherosclerosis initiation (3 fold increase in lesion size after 6 weeks) without any modification of plasmatic total cholesterol or triglyceride levels. The same experiments were performed in chimeric LDLr$^{-/-}$ mice devoid of PI3Kγ in the immune system. EP were not able to accelerate atherosclerosis in absence of PI3Kγ demonstrating the importance of this kinase in EP-induced signaling. Whereas EDPs are able to induce *in vitro* monocytes migration and ROS production through ERC, PI3Kγ$^{-/-}$ cells are not able to tranduce EPs signal.

**Conclusion :** EP binding to ERC induces atherosclerosis progression *in vivo* through PI3Kγ activation demonstrating their important role as potential therapeutical targets.

**Session IV**

# Modeling of the hemodynamics

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In the last few years, progress in computational power has made possible bio-uids simulations. These phenomena are usually very complex ones, involving di_erent scales (spatially and temporally) and combining multi-physics. The _nal objective is to obtain informations that are di_cult or even impossible to reach in vivo on patients, and thus to give clinicians modelization tools. In the sequel, we will focus on the main di_culties encountered in such simulations.
Several works on bio-uids simulation have already been done, e.g., for cardiovascular blood ows simulation [17, 10, 1, 14, 7] or air ow simulation in human lung [12, 3, 2]. Most of the e_ort on blood ows was obviously done in arteries or large vessels, as these are the easier to extract. There is still a lack of work in blood ow simulation in veins as their bio-mechanical behaviour is still not fully understood.

One of the key points in bio-uid simulation is the computational domain. For a very long time, people worked with idealized vessel or idealized geometry [20]. However, recent works have shown that these simulations are not su_cient to mimic the reality [5, 18]. So, e_ort has been made on simulations in realistic geometries, reconstructed from medical images. This part is still an obstacle as models obtained from segmentation techniques are not suitable for computational purposes. These models (made of voxel sets) only allow to obtain a surface mesh which has to be pre-processed. This step, from vascular models to computational meshes, still needs human intervention [4, 11].
Blood is composed of multiple cells and is therefore a non-Newtonian uid. But usually, this non-Newtonian behaviour is considered to only occur in small vessels. In a _rst approximation, the blood is considered as an incompressible Newtonian uid [17] in particular in large vessels. So, most of the time, simulations are made by solving the Navier-Stokes equations of the incompressible uid dynamics. Moreover, when dealing with very large vessels as the aorta for example, a simple uid simulation is not su_cient as the artery responds to the blood ow and interacts with it. Simulations were made showing that the recirculation visible in the aortic cross is no more visible without uid-structure interaction.
Another issue in bio-uid simulations is boundary conditions. For the inlet, works were done showing that using measured data on patients instead of idealized conditions are much better [19]. For the outlets, boundary conditions need to mimic the rest of the network (little arteries beyond the image resolution, veins and capillaries), which has to be cut in order to keep a reasonable simulation in terms of time and memory [8]. A solution is then to couple the full 3D simulation in the domain with less dimensional model on the outlet, such as one-dimensional ones [16, 15, 13].
Last issue concerns veri_cation and validation of bio-uids simulation. Indeed, most of the information are di_cult or impossible to obtain in vivo from the patients, hence comparison with experiments are most of time unattainable. So, to validate the work, there is a need of simpli_ed models, some measurements as velocimetry data or _nally virtual images that can be compared with the real ones [9, 6]. This is why the issue of our ANR project, named VIVABRAIN, is to develop a multidisciplinary pipeline for the generation of virtual (i.e., simulated) angiographic images (more precisely, Magnetic Resonance Angiographies, MRA) of the human brain, associated to their anatomical (3D) and hemodynamic (3D+t) models (providing ground-truths).

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**Session IV**

## QUANTITATIVE ANALYSIS OF CARDIOVASCULAR ENERGETICS

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**Abstract**

One of the aims of the quantitative analysis of cardiovascular energetics is to explain the cellular mechanisms of the metabolic aspect of Frank-Starling law – increase of the rate of oxygen consumption and energy fluxes with the elevation of left ventricle filling under conditions of the metabolic homeostasis. We analyze the recent important and remarkable advancements in studies of compartmentation of adenine nucleotides in muscle cells due to their binding to macromolecular complexes and cellular structures, which results in non-equilibrium steady state of the creatine kinase reaction. We discuss the problems of measuring the energy fluxes between different cellular compartments and their simulation by using different computer models. Energy flux determinations by $^{18}O$ transfer method have shown that in heart about 80% of energy is carried out of mitochondrial intermembrane space into cytoplasm by phosphocreatine fluxes generated by mitochondrial creatine kinase from adenosine triphosphate (ATP), produced by ATP Synthasome. We have applied the mathematical model of compartmentalized energy transfer for analysis of experimental data on the dependence of oxygen consumption rate on heart workload in isolated working heart. The analysis of these data show that even at the maximal workloads and respiration rates phosphocreatine flux, and not ATP, carries about 80–85% percent of energy needed out of mitochondria into the cytosol. Application of the Metabolic Control Analysis shows that the metabolic aspect of Frank-Starling law is explained by regulation of respiration on the beat-to-beat basis by mechanisms of local signaling within Intracellular Energetic Units.

**Session IV**

## Inorganic-phosphate-dependent autophagocytosis derangement and calcific events : Responsivity of aortic valve interstitial cells in cultures at different normophosphatemic-like conditions

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**UNDERGROUND** - Calcific aortic valve stenosis is the third cause of cardiovascular disease in the developed world with surgical valve replacement still being unavoidable because of poor prognostic capability and therapeutic effectiveness. The calcific process can take place in hyperphosphatemic conditions, metastatic calcification, as well as in normophosphatemic ones, dystrophic calcification. The conventional threshold values ascribed to inorganic phosphate concentration ([Pi]) in diagnosing normophosphatemia range between 0.8 mM and 1.45 mM to 2.0 mM [Pi]. In primary cultures mimicking metastatic calcification ([Pi]=3.0 mM) a (critical) major role was found to be played by [Pi] in priming a procalcific cell degeneration of bovine aortic valve interstitial cells (bAVICs), with mineralization enhancing subsequent to superstimulation with bacterial lipopolysaccharide (LPS) plus conditioned medium (CM) from cultured LPS-stimulated macrophages [1]. Here, bAVIC cultures containing different final [Pi] (0.8mM, 1.3 mM, and 2.0 mM) were used, thus mimicking dystrophic calcification and including borderline concentrations on respect to hypophosphatemic- and hyperphosphatemic-like conditions.

**RESULTS** - Under the inverted microscope, neither cell death signs nor appearance of calcific nodules were observed for bAVIC cultures except for those containing 2.0 mM Pi. At 3-day-long incubation, immunoreactivity to the specific marker of mature autophagosomes MAP1-LC3A was higher for control bAVICs with decrease for both Pi-cultures and PI-LPS-CM-cultures containing 0.8 mM, 1.3mM and 2.0 mM Pi in the order. For all cases there was a superimposing time-dependent decrease. Parallel immunohistochemical detection of apoptosis showed low positivity to caspase-8 and almost unreactivity for Caspase-9, caspase-3 and annexin-V. For Pi-cultures and Pi-LPS-CM-cultures containing 0.8mM Pi and, at greater extent, those containing 1.3mM Pi, bAVICs showed prominent autophagocytosis to have started and atypically progressed, with (i) a progressive RER enlargement, thereby causing cytoplasm compartmentalization into hollows or canalicular spaces which confined altered organules, and (ii) concurrent loss of autophagosomes. Conversely, in all 2.0 mM-Pi-cultures most bAVICs were affected by degenerative events as described for severe metastatic-like calcification, such as the appearance of phthalocyanin-positive material outcropping at cell surface and acting as hydroxyapatite nucleator, besides being source of real calcospherulae.

Quantitative spectrophotometric estimations of calcium amounts and alkaline phosphatase activity were consistent with the morphological data, with (i) similar values for Pi-LPS-CM-cultures *versus* Pi-cultures and control cultures, at 0.8 mM Pi and 1.3 mM Pi, and (ii) significantly higher values for Pi-LPS-CM-cultures versus Pi-cultures and these latter *versus* controls, at 2.0 mM Pi.

**CONCLUSIONS** - The differential reactivity to MAP1-LC3A suggested autophagocytosis to be an epiphenomenon which is simply related to cell survival mechanisms. The restriction of immunopositivity to caspase-8 suggests apoptosis to be not significant in promoting the calcific process. Although the finding of atypical features of autophagocytosis, no relation with calcification seems to exist, unless this process derangement may occur very fastly in the first incubation hours for 2.0 mM-Pi-cultures. Moreover, bacterial-derived inflammation seems to be regarded as an effective trigger for the higher normophosphatemic [Pi]. Interestingly, the propensity of bAVICs to undergo procalcific degeneration resulted to correlate with [Pi] in such a way that a differential discrimination of this parameter within the conventional normophosphatemic range is mandatory for a proper evaluation of the risk for dystrophic valve calcification.

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**Session V**

**Plasma lipids and the humoral response in atherogenesis**

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Atherosclerosis is a multifactorial disease and a major cause of morbidity and mortality in the western world. Lipid changes, inflammation and immune activation are the key players in its development. In the last decade, the immune processes involved have gained more relevance to the extent that atherogenesis has become recognised as an "immune disease".

Regarding the lipid profile, LDL has been elected as the prime target for treatment, mainly as a result of the efficacy of statins. However, in the last decade, HDL has been recognized as one of the most important factors in atherogenesis due to its multiple actions in blocking inflammation, oxidation and endothelium activation. As far as the immune response is concerned, most of the studies have looked at the cellular response and little is known about the humoral immune response.

In our approach to the HDL role in atherogenesis, we have addressed the importance of HDL in the direct activation of the immune system as it stabilizes APC:T cell conjugates by altering the TCR signalling kinetics. Then, we identified antibodies against HDL in patients with atherosclerosis-associated clinical events (stroke, coronary heart disease ...etc.), with and without other immune diseases.

Anti-HDL antibodies are a family of antibodies with different antigen targets in the lipoprotein complex: Apo A-I, PON, etc. We identified how different auto-antibodies directed towards the HDL components are associated with different risk factor profiles and clinical manifestations. We have also determined their capacity for directly interfering with both the inflammatory and oxidative cascades, therefore contributing to an enhanced atherogenesis.

**Session V**

## Anti mouse/human VCAM-1 nanobodies for SPECT imaging of atherosclerosis.

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**Objectives.** The inflammatory process is recognized as a major criterion for defining a vulnerable atherosclerotic plaque. As an inflammatory marker, Vascular Cell Adhesion Molecule-1 (VCAM1) therefore constitutes a relevant target for molecular imaging of such lesions. By combining nanomolar affinities and fast blood clearance, nanobodies represent potential generic radiotracers for cardiovascular molecular imaging. We aimed to generate, radiolabel and evaluate anti-VCAM1 nanobodies for noninvasive detection of atherosclerotic lesions.

**Methods.** Ten anti-mouse or anti-mouse/human VCAM1 crossreactive nanobodies with nanomolar affinities were generated, radiolabeled with technetium-99m and screened in vitro on mouse and human recombinant VCAM1 proteins and endothelial cells and in vivo in ApoE-deficient (ApoE-/-) mice.

Results. The lead compound was identified as nanobody cAbVCAM1-5. As demonstrated by surface plasmon resonance (SPR), as well as by flow cytometry, cAbVCAM1-5 was found crossreactive for mouse/human VCAM1 with Kd of 2.0±0.0 nM and 6.5±0.7 nM, respectively. In addition, it exhibited high lesion-to-control (4.95±0.85), lesion-to-heart (8.30±1.11), and lesion-to-blood ratios (4.32±0.48) ($P<0.05$ vs control C57Bl/6J mice for all 3 ratios). Atherosclerotic lesions located within the aortic arch of ApoE-/- mice were successfully identified by SPECT/CT imaging, and $^{99m}$Tc-cAbVCAM1-5 binding specificity was further demonstrated by in vivo competition experiments. Autoradiography and immunohistochemistry further confirmed cAbVCAM1-5 uptake in VCAM1-positive lesions.

**Conclusion.** The $^{99m}$Tc-labeled, anti-VCAM1 nanobody cAbVCAM1-5 allowed noninvasive detection of VCAM1 expression and displayed mouse and human crossreactivity. Therefore, this study demonstrates the potential of nanobodies as a new class of radiotracers for cardiovascular applications. The nanobody technology might evolve into an important research tool for targeted imaging of atherosclerotic lesions and has the potential for fast clinical translation.

**Session V**

**Sleep apnoea and cardiovascular consequences**.

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In the last two decades, obstructive sleep apnoea (OSA) has been identified as a common clinical condition. Epidemiological studies have confirmed a high prevalence of the disease in middle-aged adults. Upper airway collapse occurs at the pharyngeal level during sleep in OSA. The mechanisms of this pharyngeal collapse remains not fully explained. Pharyngeal neuropathy and fluid shift towards the neck have been reported in addition to the major contributions of obesity and craniofacial changes. OSA is associated with significant excessive daytime sleepiness and cognitive impairment, as well as marked cardiovascular and metabolic morbidities, leading to a significant increase in mortality. Sympathetic activation, oxidative stress and systemic inflammation have been shown as the main intermediary mechanisms associated with sleep apnoea and intermittent hypoxia (IH), the major consequence of sleep apnoea. Intermittent hypoxia has been studied both in human and animal models. There is a causal relationship that has been demonstrated between IH and several cardiovascular alterations e.g. increase in blood pressure and vascular remodelling. There are now convincing data regarding the association between hypertension, arrhythmias, stroke, coronary heart disease, increased cardiovascular mortality and OSA. There are also data in OSA and in animal models supporting the link between sleep apnoea and atherosclerosis and dysmetabolism. Whether treating sleep apnoea enables to reverse chronic cardiovascular and metabolic consequences in OSA, remains to be established in adequately designed studies, particularly in comparison with usual treatment strategies.

**Session V**

# A proteomic approach to identify circulating or tissue biomarkers of atherosclerosis

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Atherosclerosis is a multifactorial disease in which hypertension, diabetes, hyperlipidemia and other risk factors are thought to play a role. The rupture of the atherosclerotic plaque is the predominant underlying process in the pathogenesis of acute coronary syndromes and peripheral vascular disease. Mechanisms underlying plaque instability are not yet completely understood although it is generally known that it is caused by a substantial increase in proteolytic activity and inflammatory state. On the basis that plaque stability/instability could be associated with distinct patterns of protein expression; we applied a proteomic approach to study the differential protein expression in stable and unstable plaque extracts from carotid endarterectomy segments enriched in both secreted and filtered/retained proteins. We identified a panel of nine proteins, differently represented, that are indicative of a more pronounced inflammatory and oxidative status in unstable lesions respect to stable ones. Since *in situ* oxidative events could have important functional consequences on bioactivity and antigenic properties of filtered/retained proteins, we focused on protein sulfhydryl group oxidation that has been recently suggested as a possible means of redox regulation of protein function. Results from proteomics showed deep differences in oxidative state related to plaque stability. No differences were detected in the corresponding plasma samples, suggesting that the observed unbalance is an intra-plaque phenomenon.

The association between levels of specific lipoprotein classes and the risk of cardiovascular diseases is well known. We applied proteomics to characterize the apolipoprotein component of circulating lipoproteins from patients undergoing carotid endarterectomy and healthy volunteers. We identified the majority of resolved protein isoforms and detected a set of apolipoproteins differently expressed in atherosclerotic patients with respect to controls.

This method seems to be suitable to detect potential changes associated to the atherosclerotic process, and it could provide insight into the development of novel diagnostic tools and/or future therapeutic agents. Identifying changes in the 2-D apolipoprotein profile could provide insight both in the underlying mechanisms regulating the vascular tissue metabolism of lipoproteins and in the development of novel diagnostic tools and/or future therapeutic agents.

**Session V**

**Atorvastatin protects against deleterious cardiovascular consequences induced by chronic intermittent hypoxia.**

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Chronic intermittent hypoxia (IH), a major component of obstructive sleep apnea (OSA) contributes to the high risk of cardiovascular morbidity. We have previously demonstrated that IH-induced oxidative stress is involved in the hypertension and in the hypersensitivity to myocardial infarction. However, the mechanisms underlying these cardiovascular alterations are still unclear, as well as the role of potential protective treatment. Atorvastatin has pleiotropic actions, including increasing NO bioavailability and reducing inflammation and oxidative damage. The aim of this study was to evaluate the beneficial effect of a two time course of this treatment against the deleterious cardiovascular consequences of IH. Rats were divided into two groups subjected to chronic IH or normoxic (N) exposure. IH consisted of repetitive 1-min cycles (with only 30 s of a 5% inspired O2 fraction) and was applied for 8 h during daytime, for 14- (preventive protocol) or 28-days (curative protocol). Atorvastatin (10 mg.kg-1.day-1) or its vehicle was administered during the 14-days preventive protocol or the last 14 days of the curative protocol. For both protocols, systolic arterial pressure was significantly increased by 14-days IH exposure. Atorvastatin prevented this deleterious effect in the preventive protocol. Carotid artery compliance and endothelial function were significantly altered after 28-days but not after 14-days of IH exposure. Curative atorvastatin administration preserved these vascular parameters. IH also increased hypersensitivity to myocardial infarction after 14-days exposure, and atorvastatin abolished this deleterious effect. IH also enhanced cardiac NADPH expression and decreased aortic SOD activity after 14-days exposure. Atorvastatin significantly restored these activities. In conclusion, whereas IH rapidly increased blood pressure, myocardial infarction hypersensitivity and oxidative stress; compliance, endothelial function and the structural wall of the carotid artery were only altered after a longer IH exposure. Atorvastatin prevented all these deleterious cardiovascular effects, leading to a potentially novel pharmacological therapeutic strategy for OSA syndrome.