L.I.A.C

Latinorum Investigatorum de Arteriis Colloquium

The Latin Society for Vascular Research

30th LIAC Meeting on Vascular Research

Valladolid (SPAIN)

October 22th-25th, 2014

Abstract Book

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SCIENTIFIC PROGRAMME

Wednesday (Oct 22th)

17:00-18:00	Reception and documentation delivery
18:00-19:00	*OPENING SESSION:*
	Antonio Tamburro memorial lecture:
	Fernando Muñoz Box, "De la numeración romana al calendario de J. César"
19:00-20:00	**Ceremony of the 30th LIAC Anniversary:**
	"LIAC: Laboratory of innovation, 30 years of experiments and friendship", Michele Spina, Colette Lacabanne, Julia Bujan and Ida Bocchicchio
20:00	*Wine and Cheese battle*

Thursday I (Oct 23th)

SESSION I: MOLECULAR AND SUPRAMOLECULAR STRUCTURE

Chairman:

Petra Mela

09:00-09:30	Petra Mela: *"Textile-reinforced tissue-engineered cardiovascular implants"*
09:30-10:00	Israel González de Torre: "*Surface modification of biomedical devices by reactive layer by layer based on clickable elastin-like recombinamers."*
10:00-10:30	Valérie Samouillan: *"Low frequency chain dynamics of elastin-like polypeptides"*
10:30-11:00	Natalio García-Honduvilla "*Over-expression of sex hormones receptors in the insufficient varicose vein wall"*
11:00-11:30	*Coffee break*
11:30-12:00	Gabriele Nieddu: *"Proteomic analysis of plasma-purified VLDL, LDL, and HDL fractions from atherosclerotic patients undergoing carotid endarterectomy: identification of Serum Amyloid A as a potential marker"*
12:00-12:30	Alicia Fernández: "*Self-organized ecm-mimetic models based on amphiphilic multiblock bioinspired recombinamers"*

12:30-13:00 Sandra Sotomayor: "*Effects of S 42909, an NADPH oxidase inhibitor, in an experimental ischemic excisional skin wound model*"

Thursday II (Oct 23th)

SESSION II: BIOMATERIALS AND TISSUE ENGINEERING

Chairman:

Laura Cipolla

13:00-13:30	Laura Cipolla *"Smart biomaterial functionalizations for regenerative medicine*", invited speaker
13:30-14:30	*Lunch*
14:30-15:00	Michele. Spina: *"Biocompatibility evaluation of xenogenic biomaterials: distribution and quantification of residual nucleic acids and alpha-Gal epitope"*
15:00-15:30	Brigida Bochicchio "*From elastin-derived peptides to glycopeptides: self-aggregating materials as new perspectives in the treatment of cardiovascular diseases"*
15:30-16:00	*Coffee break*
16:00-16:30:	Vincenzo. La Carrubba: "*An Innovative Method to Produce Scaffolds with a Pore Size Gradient for Tissue Engineering Applications*"
21:30	*Gala Dinner*

Friday I (Oct 24th)

SESSION III: CARDIOVASCULAR SIGNALING AND MODELING

Chairman:

Hugo Alexandre Ferreira

09:00-09:30	Hugo Alexandre Ferreira, *"Advances in vascular magnetic resonance imaging",* invited speaker
09:30-10:00	Benjamin Vallin: "*Characterization of a new adenylyl cyclase 8 isoform possibly involved in vascular smooth muscle cells trans-differentiation*"
10:00-10:30	Concepción Vicenta Llorente-Cortés: *"A combined biochemical/biophysical investigation on the role of cardiomyocyte intracellular cholesteryl ester accumulation on tropoelastin"*

SESSION IV: CELL BIOLOGY AND PHYSIOPATHOLOGY

Chairman:

Marie-Paule Jacob

10:30-11:00	Marie-Paule Jacob: *"Pharmacological strategies to stimulate elastogenesis in the aorta"*, invited speaker
11:00-11:30	*Coffee break*
11:30-12:00	Alain-Pierre Gadeau: *"Nerve Desert Hedgehog knockdown induces vessel function impairment contributing to diabetic neuropathy"*
12:00-12:30	Sebastien Blaise: *"Elastin derived peptide: from insulin resistance to vascular diseases"*, invited speaker
12:30-13:00	Josune Orbe: *"Role of MMP-10 in vascular inflammation and thrombosis"*, invited speaker
13:00-13:30	Gustavo Egea: "*Vascular smooth muscle phenotypic alterations in aortic aneurysms of patients with Marfan syndrome*"
13:30-14:30	*Lunch*

Friday II (Oct 24th)

14:30-15:00	Paolo Romagnoli: "*Dendritic cell involvement in inflammatory processes leading to cardiovascular disease*", invited speaker
15:00-15:30	Muriel Laffargue: "*Absence of PI3Kγ leads to increased reendothelialization in mice through IP-10 regulation*"
15:30-16:00	*Coffee break*
15:30-16:30	Business meeting

Saturday (Oct 25th)

SESSION V: CLINICAL APPROACH

Chairman:

Henrique Silva

09:30-10:00	Francesco Serino: *"Need for a paradigm shift in the biomedical device engineering: from mechanic to synthetic biology"* invited speaker
10:00-10:30	Henrique Silva: **"***Evaluation of the peripheral microcirculation with Wavelet Transform and Detrended Fluctuation Analysis"*, invited speaker
10:30-11:00	Pablo Gallo***:*** *"As ablative radiofrequency technique in the treatment of varicose veins in the lower limbs".*
11:00-11:30	*Coffee break*
11:30-12:00	Antonietta Pepe: *"Self-assembling nanomaterials from elastin-based triblock peptides"*
12:00-12:30	Gilles Faury: "*Effects of intermittent hypoxia on aorta and vascular smooth muscle cell functions during aging"*
12:30	*Closure ceremony*

Oral Presentations

Thursday I (Oct 23th)

SESSION I: MOLECULAR AND SUPRAMOLECULAR STRUCTURE

Chairman:

Petra Mela

Textile-reinforced tissue-engineered cardiovascular implants

Petra Mela, Ricardo Moreira, Frederic Wolf, Luis Hurtado Aguilar, Maximilian Schilling,Valentine N. Gesche, Julia Frese, Stefan Jockenhoevel

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Tissue-engineered constructs serving predominantly a mechanical function, as in the case of cardiovascular implants, must possess adequate mechanical properties at the moment of implantation. Ideally tissue equivalents are developed from three-dimensional (3D) scaffolds providing the biochemical and physical environment for the development of a functional native-like tissue. Bulk hydrogels, either natural or synthetic, act as homogeneous 3D scaffolds with uniform cell distribution and high cellular viability, but they generally exhibit poor mechanical properties. Textile

reinforcement of cell seeded gels not only provides mechanical stability, but also anisotropic behaviour and the possibility of controlling the organization of the newly synthesised extracellular matrix. This abstract presents different strategies to produce cardiovascular implants based on fibre-reinforced cell seeded fibrin cardiovascular implants.

Specifically, fibrin-based heart valves were designed and realized for the pulmonary, aortic and mitral position either for surgical or minimally invasive implantation. Textile co-scaffolds were used to create biomimetic load-bearing structures and/or to confer the 3D geometry. The use of a warp-knitted scaffold enabled the realization of new semilunar valves with a tubular-leaflet design and biomimetic mitral valves which could withstand the hemodynamic conditions of the systemic circulation with only four weeks of cultivation in bioreactor systems. This time was even shortened in the case of small-calibre vascular grafts moulded using either a biodegradable or a non-biodegradable textile mesh which reached burst strengths up to 1000 mmHg after two weeks of dynamic conditioning and could be successfully implanted in the arterial circulation of the sheep.

Surface modification of biomedical devices by reactive layer by layer based on clickable elastin-like recombinamers.

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Processes that happens between the surface of the implant and the biological systems are a crucial issue that determine the fate of the implant integration. To improve the success ratio of the implant integration the Layer by Layer (LbL) technology is a good candidate, because it is an easy approach to perform a consecutive self-assembly of layers of at least two distinct materials onto substrates[1].

Recently, our group has developed a new family of hydrogels based on the crosslinking of at least two modified bioactive Elastin-like recombinamers through a catalyst free click reaction (ELR-CFCGs)[2]. In this work these materials will be applied in the LbL technique to surface modification with the idea of combining the control and efficiency of the LbL approach with the stability and robustness of the covalent bonding. Several substrates, polystyrene, titanium, glass and PS beads as simple surface systems were modified. In addition, a more complex structure, such as a coronary stent was also coated to get a continuous layer that helps to its integration after implantation. Two methodologies were employed. The first approach consists in consecutive immersions of the substrate inside two different ELRs clickable solutions, in such a way to the first solution will react forming a covalent bond with the second ELR solution (till 5 immersions on each solution). The first layer is adhered to the plasma activated

substrate by electrostatic interactions. In the second approach, an additional washing step was introduced after each layer deposition. In this way, the thickness and topography of the LbL structure can be controlled.

The surface modification was characterized by techniques as SEM, AFM, XPS, contact angle and fluorescence microscopy (the ELRs were modified to bear a fluorescence probe).

Acknowledgments:

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(1) Costa, R. R.; Mano, J. F. *Chemical Society Reviews* **2014**, *43*, 3453.

(2) González de Torre, I.; Santos, M.; Quintanilla, L.; Testera, A.; Alonso, M.; Rodríguez Cabello, J. C. *Acta Biomaterialia* **2014**, *10*, 2495.

Low frequency chain dynamics of elastin-like polypeptides

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Elastin, the protein responsible for elasticity of tissues such as lung, skin and arterial walls consists of a three-dimensional network whom turn-over is almost absent under physiological conditions. Under certain pathological conditions elastin is attacked by MMP12 releasing short polypeptides able to give rise to amyloid-like fibers. In particular conformational and microscopy studies showed that the polypeptide (S4) released during the proteolysis of human tropoelastin by MMP12 could form amyloid-like fibers in vitro.

In this study we chose to compare the chain dynamics of S4 peptides and S4 fibers in order to extract the influence of the architecture on the molecular mobility of these systems. For this purpose, we used the classical techniques devoted to the characterization of polymers and biopolymers, which have already showed their suitability for the study of native elastin, namely FTIR, Differential Scanning Calorimetry (DSC), TSC (Thermally Stimulated Currents) and Dynamic Dielectric Spectroscopy (DDS).

The S4 peptides (LVGAAGLGGLGVGGLGVPGVGG, molecular weight: 1734 g.mol^{-1}) were synthesized by solid-phase methodology, purified and freeze-dried. S4 peptides were aggregated in S4 fibers in solution at 30mg/mM under stirring at 80°C for 3 hours.

A close correlation is evidenced between the secondary structure/ultrastructure of the studied polypeptides and their chain dynamics. Peculiar thermal and dielectric signatures of the amyloid fibers can be detected both at the localized and delocalized levels in the condensed state. Dielectric experiments are peculiarly sensitive to conformational changes in these amyloidogenic peptides. These nanocrystalline materials possess several phase transitions between crystalline structures of different polarities. As shown for some model peptides, their self-assembly into amyloid fibers probably lead to macroscopic dipole moments explaining the enhancement of their dipolar properties.

Over-expression of sex hormones receptors in the insufficient varicose vein wall

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Varicose veins are the most common form of primary venous insufficiency, with a high prevalence in the western population. Data from the clinical course and epidemiology of major varicose veins of the lower limbs, suggests that sex hormones can directly influence the development of the disease though their intracellular receptors, located in cells of the venous wall.

The aim of this study was to determine the presence and localization of estrogen (ER), progesterone (PR) and androgen (AR) receptors, in the cells of normal and varicose vein wall.

In this study, samples from patients without a history of venous disease (control group: n=12) and chronic venous insufficiency (experimental group: n=12) were used. The samples were divided according to the gender of the patients. Immunohistochemical techniques were performed using monoclonal antibodies against nuclear ER, PR and

AR. Real time RT PCR techniques were used to determine the gene expression of the same receptors.

The ER and PR were localized in the nuclei of cells in the three vascular wall layers. In pathological varicose veins ER and PR were more abundant than in healthy veins, especially in women. Furthermore, AR was confined only to the nuclei of a few cells in the adventitial layer of healthy veins. In the varicose veins the positive cells were mainly located in the neointimal layer. PR mRNA expression was significantly higher in varicose veins regarding control in both gender groups. No differences in this receptor were observed between men and women. Although ER messenger in varicose veins was higher than that of controls in both groups, differences were not significant. AR mRNA expression, only in the men group was significantly higher in varicose veins, while no significant differences were observed in women.

Over-expression of sex hormones receptors in the varicose vein wall, reinforces the hypothesis about hormonal involvement in the development of this type of vascular pathology.

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Proteomic analysis of plasma-purified VLDL, LDL, and HDL fractions from atherosclerotic patients undergoing carotid endarterectomy: identification of Serum Amyloid A as a potential marker

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Apolipoproteins are a very heterogeneous protein family, implicated in plasma lipoprotein structural stabilization, lipid metabolism, inflammation, or immunity. Obtaining detailed information on apolipoprotein composition and structure may contribute to elucidating lipoprotein roles in atherogenesis and to developing new therapeutic strategies for the treatment of lipoprotein associated disorders. This study aimed at developing a comprehensive method for characterizing the apolipoprotein component of plasma VLDL, LDL, and HDL fractions from patients undergoing carotid endarterectomy, by means of two-dimensional electrophoresis (2-DE) coupled with Mass Spectrometry analysis, useful for understanding plaque biology, and identifying potential markers of plaque progression (presence and vulnerability). The adopted method allowed obtaining reproducible 2-DE maps of exchangeable apolipoproteins from VLDL, LDL, and HDL. Twenty-three protein isoforms were identified by peptide mass fingerprinting analysis. Differential proteomic analysis allowed for identifying increased levels of acute-phase serum amyloid A protein (AP SAA) in all lipoprotein fractions, especially in LDL from atherosclerotic patients. Results have been confirmed

by western blotting analysis on each lipoprotein fraction using apo AI levels for data normalization. The higher levels of AP SAA found in patients suggest a role of LDL as AP SAA carrier into the subendothelial space of artery wall, where AP SAA accumulates and may exert noxious effects.

Self-organized ecm-mimetic models based on amphiphilic multiblock bioinspired recombinamers.

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Although significant progress has been made in the area of injectable hydrogels for biomedical applications and model cell niches, further improvements are still needed, especially in terms of mechanical performance, stability and biomimicry of the native fibrillar architecture found in the extracellular matrix (ECM).

This work focuses on the design and production of bioinspired protein-based biopolymers able to spontaneously form stable physical hydrogels under physiological conditions [1]. For such purpose, we have fixed our attention on several self-assembled motifs present in Nature, such as elastin-like motifs, silk-like moieties and leuzine zipper domains, which are reported to adopt β-turns, β-sheet, α-helix conformations respectively. Such motifs have been combined in a carefully designed manner thanks to the DNA technology, resulting in the creation of multiblock bioinspired polymers, with an absolute control over their sequence, named as recombinamers [2]. The composition of each recombinamer has been dictated by a reductionist approach, in which the minimal structure displaying the desired physical properties is the subject of study [3]. In all cases, the dynamics of gelation, the mechanical properties of the thus-formed

hydrogels, and the structural characteristics at a microscopic and molecular level were studied.

The results point to the huge potential of these systems as a basis for the development of injectable biomaterial platforms towards a fully functional, biomimetic, artificial extracellular matrix and cell niches.

Acknowledgments:

THE GRAIL Project, Grant number: 278557

[1] Zhao, Y., Sakai, F., Su, L. Liu, Y., Wei, K., Chen, G., Jiang, M. (2013). Progressive macromolecular self-assembly: from biomimetic chemistry to bio-inspired materials. Adv. Mater. 25, 5215−5256.

[2] Girotti, A., Fernández-Colino, A., López, IM., Rodríguez-Cabello, JC., Arias, FJ. (2011) Elastin-like recombinamers: biosynthetic strategies and biotechnological applications. Biotechnol J. Oct;6(10):1174-86

[3] Fernández-Colino, A. Arias, F. J., Alonso, M. and Rodríguez-Cabello, J.C. (2014). Self-Organized ECM-Mimetic Model Based on an Amphiphilic Multiblock Silk-Elastin-Like Corecombinamer with a Concomitant Dual Physical Gelation Process. Biomacromolecules. DOI: 10.1021/bm501051t.

Effects of S 42909, an NADPH oxidase inhibitor, in an experimental ischemic excisional skin wound model

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Leg ulcer affects about 1% of individuals during their lifetime and despite compression therapy, considered as the first line treatment, 30% of patients remain unhealed after 1 year. Persistent tissue ischemia is an important underlying feature of chronic wounds that severely impairs the healing process. The aim of this study was to evaluate in an experimental ischemic excisional skin wound model, the effect of systemic therapy with S 42909, an inhibitor of NADPH oxidase activity, which possesses vascular anti-inflammatory properties by preventing leukocyte-endothelial cell adhesion and modulates the redox-environment.

Ischemia grade 3 was induced on right ears of male New Zealand rabbits. 24 h later, an excisional wound of 2 cm diameter was performed. S 42909 at 10 and 30 mg/kg (n=3 each) was administered orally by gavage after induction of the lesion. Ischaemic control and treated groups were sacrificed on day 14. Macroscopic evaluation, contraction, reepithelialisation and histological (Masson's trichrome and hematoxiline-eosine staining) analysis were performed.

S 42909 at both doses tended to increase reepithelialisation area (about 90%) when compared with the control ischemic group. Wound contraction was lower after S 42909 treatment whatever the dose. In both treated groups, keratinocytes of the epidermis migrate in a centripetal fashion towards the wound center, showing the majority of the samples a complete reepithelialization. Neovascularization of the new formed dermal tissue was correct in both treated groups.

S42909 improved wound healing in ischemic rabbit ear ulcer as suggested by a trend to an increase in reepithelialisation, indicating the deleterious role played by excessive inflammation and oxidative stress on tissue repair. In addition, S 42909 significantly prevented wound contraction which could be deleterious for healing process. This treatment may be a promising therapy to treat skin defects.

Thursday II (Oct 23th)

SESSION II: BIOMATERIALS AND TISSUE ENGINEERING

Chairman:

Laura Cipolla

Smart biomaterials functionalization for regenerative medicine

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The integration of biological extracellular matrix (ECM) components and synthetic materials is a promising pathway to fabricate the next generation of biomaterial scaffolds that more accurately emulate the microscale heterogeneity of natural ECM. Research on nanostructured biomaterials surface functionalisation has become one of the hottest topics in biomaterials for regenerative medicine. The chemistries used for matrix mimic synthesis and functionalization must to be easy to control and biocompatible.

Since cell contact with the biomaterial surface is a key point, in recent years, biomaterial design has focused on the exposition and incorporation of signalling molecules into scaffold materials. Carbohydrates are well-known to have a wide variety of biological functions, participating in a number of recognising. Thus, it is clear that carbohydrates may be used in the bioactivation of material surfaces toward tissue engineering applications.

Innovative and recent examples of material functionalisation for tissue engineering applications with signalling and relevant glycidic scaffolds will be outlined. Particular attention will be drawn to the chemistry used for covalent attachment of relevant carbohydrates to materials of different chemical nature[1]. Preliminary biological assays of these biomaterials as candidates for tissue regeneration will be presented[2].

References

1. Taraballi, F.; Russo, L.; Battocchio, C.; Polzonetti, G.; Nicotra, F.; Cipolla, L. A model study for chemoselective tethering of (bio)active molecules to biomaterial surfaces through arginine. Org Biomol Chem 2014; 12:4089-92.
2. Russo, L.; Sgambato, A.; Lecchi, M.; Pastori, V.; Raspanti, M.; Natalello, A.; Doglia, S.M.; Nicotra, F.; Cipolla, L. Neoglucosylated collagen matrices drive neuronal cells to differentiate. *ACS Chem Neurosci* 2014; 5(4):261-5.

Acknowledgements.

The work has been supported by Fondazione Cariplo, grant n° 2008-3175, 2010-0378 and 2011-0270, and PRIN 2011 2010L9SH3K

Biocompatibility evaluation of xenogenic biomaterials: distribution and quantification of residual nucleic acids and alpha-Gal epitope

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Decellularized xenogeneic scaffolds are currently employed for the healing of diseased tissues. However their use is permitted even without a quantitative assessment of cell material elimination, like the alpha-Gal epitopes. Moreover, cell removal procedures are not monitored to prove the elimination of the calcific potential associated to the nucleic acids remnants. The current treatment with glutaraldehyde is reducing but it is not eliminating the immunogenicity of implanted xenogeneic tissues as particularly for the alpha-Gal epitopes. Recent studies have reported investigations on the biocompatibility evaluation of xenogeneic bioprosthetic devices. Here we report results concerning the alpha-Gal epitopes and nucleic acid detection in novel xenogeneic bioprosthetic preparations that have shown promising preclinical/clinical results.

The alpha-Gal quantification was carried out by an ELISA test developed by our group and based on the use of the monoclonal anti apha-Gal antibody M86. Immunofluorescence analysis was performed for the visual distribution of both xenogeneic epitopes and nucleic acids residues. A commercially available kit was adopted for total DNA quantification. The amount and distribution of the alpha-Gal epitopes resulted different between the investigated biomaterials, while the nucleic

acid remnants appeared as a common feature, even in those biomaterials indicated by the manufacturer as free from cell remnants.

Insufficient evaluations of the residual content of xenogeneic epitopes, detergents and nucleic acid have led to disappointing and disastrous results. The risk of these dramatic accidents reoccurrence remains very high unless safety parameters, like the complete removal of major xenogeneic determinants and of nucleic acid are adopted in the manufacturing practices.

From elastin-derived peptides to glycopeptides: self-aggregating materials as new perspectives in the treatment of cardiovascular diseases

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The laboratory of bioinspired materials is involved in the synthesis of polypeptides inspired to elastomeric proteins since many years. The final aim of the research is to use them as scaffolds in tissue engineering. More recently, the DNA recombinant technologies let us the production of high molecular weight polypeptides. The cross-linked polypeptides gave rise to 2/3-D supramolecular structures. Recently, we focused our attention on bioconjugation products obtained by using as starting materials carbohydrates and elastin-derived peptides. The reactions were carried out in mild conditions (aqueous environment, room temperature, physiological pH) and gave rise to self-aggregating glycopeptides. Preliminary results suggest them as good candidates as biomaterials and as drugs in the prevention of surgical site as drugs in the prevention of surgical site.

An Innovative Method to Produce Scaffolds with a Pore Size Gradient for Tissue Engineering Applications

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Thermally Induced Phase Separation (TIPS) is a technique for the production of porous scaffold for Tissue Engineering applications. A wide range of microporous morphologies, in terms of pore size and distribution, can be obtained by tuning TIPS processing parameters, especially thermal history. The production of scaffolds for bone tissue regeneration is a challenging target: as a matter of fact, scaffolds must mimic the bone morphology, thus requiring a gradient of pore dimension and morphology along one dimension.

To attain this goal, an experimental apparatus capable to impose different thermal histories on the two sides of a sample was designed, set up and tested. The sample (35x35 mm surface, 10 mm thickness) was located between two Peltier cells, employed to control temperature on sample surfaces. In that way each sample surface can be thermally controlled independently and anisotropic scaffolds were produced by following various thermal protocols on both sample surfaces. The system investigated in this study is the ternary solution poly-L-lactic acid (PLLA)-dioxane (solvent)-water (non-solvent). The as-obtained foams were inspected by Scanning Electron Microscopy (SEM), to verify the pore size and distribution.

The results showed that via this technique, based on TIPS, is possible to produce scaffolds with a pore size increasing along sample thickness. The obtained pore dimension on one side of the sample was about 70 micron, whereas it was around 240

micron on the opposite surface. By moving along the sample thickness, the pore dimension increased steadily. Moreover, since pure PLLA does not show fully satisfactory mechanical properties for bone tissue engineering purposes, composite PLLA/hydroxyapatite (HA) scaffolds were produced, in order to improve foams' mechanical performances. Compression tests showed a fourfold increase of Young module with respect to pure PLLA scaffold.

All things considered, a reliable route for the production of scaffolds with a controlled pore size distribution was assessed, thus offering a valid support to tissue engineering applications, such as bone tissue engineering.

References

1. F. Carfì Pavia, V. La Carrubba, S. Piccarolo, V. Brucato. J Biomed. Mater. Res. Part A 86, 2008, 456-466

Friday I (Oct 24th)

SESSION III: CARDIOVASCULAR SIGNALING AND MODELING

Chairman:

Hugo Alexandre Ferreira

Advances in vascular magnetic resonance imaging

Hugo Alexandre Ferreira

In this talk, an overview of vascular magnetic resonance imaging (VMRI) techniques and applications, and advances in the field will be given. First, VMRI techniques will be presented and classified, and their physical principles will be described succinctly. Then, an explanation of how these techniques can be used to study the vascular system from multiple spatial (from capillaries to large vessels) and temporal scales (from steady-state to dynamic flow) will follow. Later, molecular MRI methods to characterize the vessel wall and to assess vascular-related gene expression will be described. Finally, a glimpse into the future will be given, stating the importance of vascular research in MRI-guided stem-cell therapeutics and nanorobotics.

Characterization of a new adenylyl cyclase 8 isoform possibly involved in vascular smooth muscle cells trans-differentiation

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Trans-differentiation of vascular smooth muscle cells (VSMC) from a contractile/quiescent to a migratory/proliferative/secretory phenotype is a critical step in atherosclerotic lesion development and post-angioplasty restenosis *(Heusch G. et al., Lancet, 2014)*. Furthermore, cyclic adenosine monophosphate (cAMP) plays a major role in the control of VSMC phenotype *(Li R. et al., Circ Res., 2004)*; its compartimentalization into the cell is achieved by adenylyl cyclases (ACs) and phosphodiesterases (PDEs), also responsible for cAMP production and degradation respectively. Consistently, we showed that VSMC trans-differentiation is partially related to the *de novo* expression of adenylyl cyclase isoform 8 (AC8). Indeed, AC8, detected in trans-differentiated VSMC of human atherosclerotic carotids, is required *in vitro* for interleukine-1β-induced VSMC transformation into migratory/inflammatory cells *(Gueguen et al., J Pathol., 2010)*. We now extended our previous results evidencing that more than 80% of AC8 transcripts expressed in IL-1β-treated rat VSMC are composed of a spliced variant encoding a truncated form of AC8 lacking the first five trans-membrane domains. This corresponds to an in-frame deletion of amino acids 180 to 317. Biochemical analysis indicated that this deletion has no incidence on AC8 membrane localization but leads to a switch of the N terminus domain from intra- to extra- cellular localization. Because this region is of great importance in regulating AC8 activity *(Willoughby D. et al., J Cell Sci., 2012)*, such a conformational change could radically modify AC8 function and

regulation. This is consistent with cAMP dynamics measurements performed using FRET-based biosensors. Indeed, while the overexpression of AC8 “full length” in HEK causes a dramatic increase in basal cAMP concentration, that of AC8Del180-317 did not; alternatively, AC8Del180-317 triggers a profound reconfiguration of induced-cAMP dynamics resulting from a unique increase in PDE4 activity. Because i) an identical reconfiguration is observed in IL-1β-treated VSMC and ii) PDE4 is involved in inflammation and migratory process in various cell types *(Houslay MD., Mol Pharmacol., 2005)*, we suggest that AC8Del180-317 participates to rat VSMC trans-differentiation through PDE4. Further investigations will clarify the potential role of AC8Del180-317 in pathological vascular remodeling and evaluate its relevance as a potential therapeutic target.

A combined biochemical/biophysical investigation on the role of cardiomyocyte intracellular cholesteryl ester accumulation on tropoelastin.

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Dyslipemia has a direct impact on cardiac remodeling by altering extracellular matrix (ECM) components. One of the main ECM components is elastin, a proteic three-dimensional network that can be efficiently degraded by cysteine proteases o cathepsins. Dyslipemic status in insulin resistance and combined hyperlipoproteinemia diseases include raised levels of very low density lipoproteins (VLDL), a triglyceride (TG)-cholesterol ester (CE)-rich lipoprotein. Enhanced VLDL concentration promotes cardiomyocyte intracellular cholesteryl ester (CE) accumulation in a LRP1-dependent manner. The aim of this work was to analyze the effect of cardiomyocyte intracellular CE accumulation on tropoelastin (TE) characteristics and to investigate the role of LRP1 and Cathepsin S (CatS) on these effects. Molecular studies showed that LRP1 deficiency impared CE selective uptake and accumulation from TG-CE- rich lipoproteins (VLDL+IDL) and CE-rich lipoproteins (aggregated LDL, agLDL).

Biochemical and confocal microscopic studies showed that LRP1-mediated intracellular CE accumulation increased CatS mature protein levels and induced an altered intracellular TE globule structure. Biophysical studies evidenced that LRP1-mediated intracellular CE accumulation caused a significant drop of Tg2 glass transition temperature of cardiomyocyte secreted TE. Moreover, CatS deficiency prevented the alterations in TE intracellular globule structure and on TE glass transition temperature. These results demonstrate that LRP1-mediated cardiomyocyte intracellular CE accumulation alters the structural and physical characteristics of secreted TE through an increase in CatS mature protein levels. Therefore, the modulation of LRP1-mediated intracellular CE accumulation in cardiomyocytes could impact pathological ventricular remodeling associated with insulin-resistance and combined hyperlipoproteinemia, pathologies characterized by enhanced concentrations of TG-CE rich lipoproteins.

Chairman:

Marie-Paule Jacob

Which strategy to stimulate elastogenesis in the aorta?

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Rationale: In vascular pathologies like supravalvular aortic stenosis, aneurysmal disease, atherosclerosis and during aging, arterial elastin content is diminished by decreased elastin synthesis and/or degradation of elastic fibers. Describe mechanisms implicated in elastin synthesis would be of interest to find new elastogenic molecules to treat those pathologies.

Objective: We first analysed the signaling pathway linking calcium concentration to elastin regulation. Then, using inhibitors of the pathway, we investigated in vitro and in vivo their elastogenic ability.

Methods and Results: The Brown Norway rat strain was used in this study as an arterial elastin deficient model. Our data indicate that A23187, a calcium ionophore, decreases elastin expression in vascular smooth muscle cells, both transcriptionally and post-transcriptionally. The addition of A23187 induces transient activation of ERK1/2, leading to an upregulation of AP1 transcription factors, which correlates with the inhibition of elastin gene transcription. Pre-treatment with U0126, an inhibitor of ERK1/2 phosphorylation, abolished the inhibition of elastin gene transcription by A23187. In vitro, U0126 increases elastin synthesis and in vivo, 24 hours after an

intravenous administration, elastin gene transcription and elastin mRNA level are increased in the rat aorta. A chronic treatment, diffusing U0126 for 10 weeks, increased aortic elastin content without changing cell number and collagen content.

Conclusions: We have shown that calcium ionophore represses elastin gene transcription via activation of ERK1/2 pathway and AP1 transcription factors. We provide strong evidence that inhibition of ERK1/2 pathway induces an increase in elastin synthesis and thus could be suitable for treating vascular pathologies characterized by diminished arterial elastin content.

Results from this study will be compared to those obtained after treatment of BN rats with potassium channel openers. Other potential therapeutic strategies will be discussed.

Nerve Desert Hedgehog knockdown induces vessel function impairment contributing to diabetic neuropathy

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*Background***:** Diabetic neuropathy, which leads to a serious decrease in the quality of life of patients, is classified among diabetes-associated microangiopathies; nevertheless the contribution of neuropathy-associated microangiopathy to the development of the disease has not been fully demonstrated.

*Methods and results***:** In the present study, we have investigated the role of Desert Hedgehog (Dhh), a gene down-regulated in diabetic peripheral nerves, in the development of neuropathy-associated microangiopathy and its contribution to the pathogenesis of the neuropathy. We have evaluated two parameters of the neuropathy: the blood nerve barrier (BNB) by measuring the diffusion of Evans blue dye around nerve capillaries and the nerve sensitivity by compartmental tests (Plantar and von Frey Hayflick tests).

We found that the endoneurial vasculature of Dhh deficient mice and type 2 diabetic $Lepr^{db/db}$ mice are impaired and more precisely have in common to be abnormally permeable and infiltrated by macrophages. Moreover, we demonstrated *in vivo* and *in vitro* that Dhh promotes the tight junction protein Claudin5 expression in endothelial

cells and that consequently Dhh knockdown is sufficient to induce blood nerve barrier (BNB) breakdown. Furthermore, we found that impaired Dhh signaling in endothelial cells is sufficient to induce hypoalgesia.

Conclusions: In conclusion, the present work demonstrates the crucial role of Dhh in maintaining BNB integrity and demonstrates for the first time that microangiopathy is sufficient to induce neuropathy.

Elastin derived peptide: from insulin resistance to vascular diseases

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In Europe, more than 55 million of patients suffer from type 2 diabetes (T2D). The WHO estimates that in 30 years, more than 66 million people will be affected by this disease. Real public health problem, the T2D is a major risk factor for cardiovascular diseases such as atherosclerosis characterized by lipid accumulations and elastin degradation into elastin derived peptides (EDP). The EDP influence the cell physiology by activation of the elastin receptor complex (ERC). During the last decade, our team has deciphered the signaling pathways triggered by the ERC upon its binding to the canonical human sequence VGVAPG. This receptor consists of the elastin binding protein, a protective protein cathepsin A and the neuraminidase-1 (Neu-1). Our data have shown that EDP signaling depends on the cell type and requires the catalytic activity of the Neu-1 subunit. Using in vivo, in vitro and in silico approaches, we demonstrate that EDP signaling could be one of the important events governing, in part, the complications linked to ageing in the cardiovascular continuum. Namely, our data show that EDP promote insulin resistance and atherosclerosis, modulate hemostasis and thrombosis, and involve the elastin receptor complex and its Neu-1 subunit. Finally, the EDP/ERC should now be considered as a marker for the development of age-oriented therapeutic approaches aiming at limiting the deleterious effects of EDP.

Role of MMP-10 in vascular inflammation and thrombosis

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Matrix metalloproteinases (MMPs) are a large family of zinc-containing endopeptidases, which contribute to the development of unstable plaques and vascular remodelling by degrading extracellular and non-extracellular matrix components (ECM). MMP-10 or stromelysin-2 is a secreted MMP with functions in skeletal development, wound healing, and vascular remodelling. MMP-10 degrades multiple components of the ECM or stromal connective tissue, such as proteoglycan, laminin, fibronectin, and collagen III and IV although this list is expanding with new non-ECM components. Multiple evidences indicate that MMP-10 is involved in vascular development and atherogenesis and contribute to plaque rupture and thrombus. It is induced by inflammatory stimuli, and it is over-expressed in atherosclerotic lesions mainly in endothelial cells and in monocyte/macrophage rich areas where it could play a detrimental role after plaque disruption. Additionally, higher MMP-10 serum levels have been associated with inflammatory markers, increased carotid intima-media thickness and the presence of atherosclerotic plaques. Therefore, MMP-10 reveals as a potential biomarker and a key molecule in atherosclerotic vascular remodelling.

To go deeper into the role of MMP-10 in the pathogenesis of atherosclerosis, genetically-modified double knockout ApoE-/- ; Mmp10-/- mice have been generated. MMP-10 deficiency resulted in a substantial reduction in atherosclerotic lesion size, increase vascular smooth muscle cell/macrophage ratio and decrease plaque

calcification, leading to a more stable phenotype. Therefore, MMP-10 plays a causal role in atherosclerosis, favouring plaque development and calcification.

Atherosclerotic plaque disruption and subsequent thrombosis formation is responsible for many thrombotic complications in cardiovascular disease. After plaque rupture, local inflammation leads to exposure of tissue factor, initiating thrombin generation. Thrombin induces endothelial MMP-10 mRNA and protein levels, through a protease-activated receptor-1 (PAR-1)-dependent mechanism. Interestingly, circulating MMP-10 levels are augmented in patients with endothelial activation associated with high (disseminated intravascular coagulation) and moderate (previous acute myocardial infarction) systemic thrombin generation. Moreover, combination of thrombin with CD40L (an inflammatory stimulus) elicits a strong synergistic effect on endothelial MMP-10 expression and increases the number of microparticles containing MMP-10 in vitro and in vivo. All these results may indicate a possible role of MMP-10 in pathological conditions associated with enhanced thrombin generation.

Progression of human vascular pathology from atherosclerosis, thrombus formation and dissolution, and therapeutic interventions led us to investigate the possible role of MMP-10 in thrombus lysis on the basis of the cooperation between the fibrinolytic and matrix metalloproteinase (MMP) systems. Besides MMPs activation by the plasminogen/plasmin system, several studies have suggested that multiple MMPs may participate in the dissolution of fibrin deposits by targeting fibrin(ogen). In vitro, we have shown that MMP-10 is capable of enhancing t-PA-induced fibrinolysis via TAFI inactivation- mediated mechanism. Moreover, a novel profibrinolytic role for MMP-10 in experimental ischemic stroke has been described, since therapeutic administration reduces reperfusion time and infarct size to the same extent as t-PA and is associated with shorter bleeding time and no intracranial haemorrhage.

In conclusion, MMP-10 plays a crucial role in vascular inflammation, atherosclerosis progression, plaque rupture and thrombus formation and resolution. Besides its role in the degradation of ECM, it can also represent a new actor in vascular fibrinolysis. The modulation of MMP-10 activity using drugs that affect the expression and function of this protein will provide new pathways for innovative therapeutic strategies in vascular pathologies.

Vascular smooth muscle phenotypic alterations in aortic aneurysms of patients with Marfan syndrome

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Objective: Marfan syndrome is characterized by the formation of ascending aortic aneurysms resulting from altered assembly of extracellular matrix microfibrils and chronic TGF-β signaling. TGF-β is a potent regulator of the vascular smooth muscle cells (VSMC) phenotype. We hypothesized that as a result of the chronic TGF-β signaling, VSMC alter their basal differentiation phenotype, which could facilitate the formation of aneurysms. This study examines whether MFS entails a TGF-β dependent phenotypic alteration of VSMC, and explores involved mechanisms at the subcellular level.

*Approach and Results***:** Immunohistochemical and Western blotting analyses of dilated aortas from Marfan patients showed overexpression of contractile protein markers (α-SMA, smoothelin, SM22α and calponin-1) and extracellular matrix proteins (collagen I) in comparison with healthy aortas. VSMC explanted from Marfan aortic aneurysms showed increased *in vitro* expression of these phenotypic markers and also of

myocardin, a transcription factor essential for VSMC-specific differentiation. These alterations were reduced after pharmacological inhibition of the TGF-β pathway. Marfan VSMC in culture showed more robust actin stress fibers and enhanced RhoA-GTP levels, which was accompanied by increased focal adhesion components and a higher nuclear localization of myosin-related transcription factor-A (MRTF-A). Marfan VSMC and *in vitro* produced extracellular matrix (ECM) measured by atomic force microscopy were both stiffer than their respective controls. **Conclusions:** In Marfan VSMC, under the upregulation of TGF-β, overexpressed contractile and ECM proteins and increased collagen I secretion results in greater cellular and ECM stiffness, which together contributes to the known aortic rigidity that precedes and/or accompanies aneurysm formation.

*Equally contributed. This work is supported by grants from MINECO BFU2012-33932, National Marfan Foundation and Fundación Ramon Areces

Inflammatory mechanisms in the pathogenesis of cardiovascular disease

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Dendritic cells (DCs) of the immune system are present in the normal human arterial wall and increase in atheroma, and appear in the arteries of laboratory rodents in experimental atherogenesis and upon traumatic injury. Rosiglitazone reduces the inflammatory response to experimental arterial injury and the intercellular contacts of DCs in this condition.

Dendritic cells can differentiate from monocytes and haematopoietic stem cells, including CD133+ highly immature ones. Rosiglitazone promotes the differentiation of Langerhans cells and inhibits that of other DC types from CD133+ hematopoietic precursors[1].

In vitro experiments on human cells[2] have demonstrated that monocyte derived DCs adhere to vascular smooth muscle cells (VSMCs) with reciprocal stimulation: DCs are induced to mature and VSMCs are induced to increase migratory activity. Adhesion is enhanced by stimulation of VSMCs with IFN-gamma and TNF-alpha and is inhibited by pretreatment of those cells with rosiglitazone and atorvastatin. The increased adhesion is mediated by CD54(ICAM-1)/CD11c and CD106(VCAM-1)/CD28; the cell

interaction is mediated also by soluble molecules, including TNF-alpha secreted by DCs, IL-6 secreted by VSMCs and MCP-1 secreted by both cell types. In an *in vivo* rat model it has been shown that adhesion between DCs and VSMCs occurs also during the response to experimental carotid injury[2]. Preliminary evidence in this respect has been found also for human atheroma[3].

Therefore DCs may be candidate to a major role in the onset and progression of vascular inflammation, even independent of specific immune responses, and may be target for therapy.

References

1. Bonetti MI *et al*. Rosiglitazone promotes the differentiation of Langerhans cells and inhibits that of other dendritic cell types from CD133 positive hematopoietic precursors. Histol Histopathol 2014; 29: 323-32.

2. Paccosi S *et al*. Stimulatory interactions between human coronary smooth muscle cells and dendritic cells. PLOS ONE 2014; 9: e99652.

3. Bacci S *et al*. Smooth muscle cells, dendritic cells and mast cells are sources of TNFalpha and nitric oxide in human carotid artery atherosclerosis. Thromb Res 2008; 122: 657-67.

Absence of PI3Kγ leads to increased reendothelialization in mice through IP-10 regulation.

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Endothelium plays a key role in arterial pathologies, and disrupt of this function is associated with some complications of atherosclerosis treatment. Indeed the use of Drug Eluting Stent (DES) coated with antiproliferative drugs to prevent intimal hyperplasia clearly interferes with correct endothelial healing leading increasing frequency of late thrombosis and the risk of neo-atherosclerosis. Thus, alternative specific targets need to be identified to prevent intimal hyperplasia without disturbing reendothelialization.

Previous results indicate that phosphoinositide 3-kinase gamma (PI3Kγ), an immuno-modulator, is an interesting target since its blockade leads to reduced atherosclerosis (1) and intimal hyperplasia (2). In addition to these effects, we now demonstrate that loss of PI3Kγ activity leads to increased endothelial coverage. By using an original mice model expressing an inactive form of PI3Kγ, we showed that arterial healing after endovascular mechanical injury of the carotid artery was increased compared to wt mice. Bone marrow transfer experiment pointed out a specific involvement of PI3Kγ activity in the medullar compartment. A screen at genetic and protein levels showed an

increase in the expression and secretion of IP-10 (IFNγ-induced protein 10) in wt injured carotid arteries compared to controlateral uninjured vessels. This increase was strongly alleviated in the absence of PI3Kγ activity suggesting a possible involvement of IP-10 in the blockade of reendothelialisation. Administration of IP-10 to mice subjected to arterial denudation decreased arterial healing whereas injection of IP-10 blocking antibody leads to accelerated reendothelialization demonstrating the role of IP-10 in this process. A comparison of endothelial healing mechanisms after mechanical and electrical injury permitted to suggest that smooth muscle cells were involved in this inflammatory response. These data demonstrate for the first time a causal link between endothelial healing and immuno-inflammatory processes and definitively place PI3Kγ as a therapeutic target to prevent intimal hyperplasia while accelerating reendothelialization.

(1) Smirnova NF, Gayral S, Pedros C, Loirand G, Vaillant N, Malet N, Kassem S, Calise D, Goudounèche D, Wymann MP, Hirsch E, Gadeau AP, Martinez LO, Saoudi A, Laffargue M. Targeting PI3K□ activity decreases vascular trauma-induced intimal hyperplasia through modulation of the Th1 response. J Exp Med. 211(9):1779-92 (2014).

(2) Fougerat A, Gayral S, Gourdy P, Schambourg A, Rückle T, Schwarz MK, Rommel C, Hirsch E, Arnal JF, Salles JP, Perret B, Breton-Douillon M, Wymann MP, and Laffargue M. Genetic and pharmacological targeting of Phosphoinositide 3-Kinase-{gamma} reduces atherosclerosis and favors plaque stability by modulating inflammatory processes. Circulation. 117(10): 1310-1317 (2008).

Saturday (Oct 25th)

SESSION V: CLINICAL APPROACH

Chairman:

Henrique Silva

Need for a paradigm shift in the biomedical device engineering: from mechanic to synthetic biology

Francesco Serino

Evaluation of the peripheral microcirculation with Wavelet Transform and Detrended Fluctuation Analysis

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Skin microcirculation provides useful data regarding the physiological mechanisms of blood flow control1. Laser Doppler Flowmetry (LDF) is a noninvasive and sensitive technique to study skin microcirculation, providing a non-stationary signal which can be simplified with tools such as the wavelet transform and the detrended fluctuation analysis (DFA). The wavelet transform fractions the LDF signal into its well-defined, main frequency components: heart, respiration, myogenic, sympathetic and metabolic2. DFA allows the determination of the α coefficient, whose value can translate correlations within a signal3. Our objective was to characterize the LDF signal obtained during a classic suprasystolic lower limb occlusion procedure with these analysis tools. A group of 30 subjects (22.3 ± 3.1 years old) was analyzed. Local blood flow was analyzed on the plantar aspect of the second toe. Descriptive and nonparametric tests were applied. Results reveal that the LDF signal is predominantly of sympathetic and endothelial origin and has different correlation profiles during the different phases of the procedure. The components' amplitude ratios and α exponents changed significantly during occlusion. The heart and respiration components showed 1/f noise-like behavior and the myogenic, sympathetic and metabolic components showed Brownian noise-like

behavior. Wavelet and DFA show promise as complementary analysis tools, helping to characterize the LDF signal, and could distinguish between physiological from medical conditions.

References

1. Holowatz, LA, Thompson-Torgerson CS, Kenney, WL. The human cutaneous circulation as a model of generalized microvascular function. J Appl Physiol 2008; 105(1):370-2.

2. Landsverk SV, Kvandal P, Bernjak A, Stefanovska A, Kirkeboen KA. The effects of general anesthesia on human skin microcirculation evaluated by wavelet transform. Anesth Analg 2007; 105(4):1012-9.

3. Peng CK, Havlin S, Stanley HE, Goldberger AL. Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. Chaos. 1995;5(1):82-7.

Self-assembling nanomaterials from elastin-based triblock peptides

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The self-assembly of biocompatible molecules, such as peptides and proteins, into regular supramolecular structures has important implications. Such biomimetic systems are becoming important for the delivery of drugs, genes, and proteins. Peptide-based nanostructures have attracted considerable attention owing to their biocompatibility, capability of molecular recognition, and well-defined structures.

Among the peptides able to self-assemble, elastin-related polypeptides represent a special group because of their simple design, their responsiveness to temperature and remarkable mechanical properties. These peculiar features of elastin-related polypeptides render them a special subject of interest as bio-nanomaterial with "smart" behavior.

In the present report we show the results of the biophysical characterization of self-assembled nanostructures formed by designed peptide sequences, based on a three-block structure.

The peptide block sequences are inspired by two proteins able to self-assemble in peculiar nanostructures of different nature, elastin and human Serum amyloid A (hSAA1) protein. The elastin sequences belong to crosslinking or hydrophobic domains of human tropoelastin, while the hSAA1 sequences are rich in phenylalanines.

The results reveal important aspects regarding conformation determinants in the nanostructure assembly of these peptides. In particular, they evidence a predominant role for the aromatic interactions. The knowledge of the subtle role of conformation in

defining self-assembly or vice versa, could help in designing building blocks for predictable nanostructures.

Acknowledgment: The financial support from MIUR (PRIN 2010-Project 2010L(SH3K)) is gratefully acknowledged.

References

1. A. Lakshmanan, S. Zhang and C. A.E. Hauser. Short self-assembling peptides as building blocks for modern nanodevices. Trends in Biotechnology 2011, 30, 155-165.

Effects of intermittent hypoxia on aorta and vascular smooth muscle cell functions during aging

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Sleep apnea syndrome (SAS), featuring intermittent respiratory interruptions during sleep, is an important public health problem since its prevalence can reach 10% of the population. The intermittent hypoxia (IH) generated by SAS is frequently associated with cardiovascular morbidity, such as hypertension, cardiac infarct and insufficiency, vascular remodeling and arterial stiffening. Furthermore, the prevalence of SAS increases with age, reaching up to 20% in the elderly. The effects of IH during aging are controversial, especially regarding the associated cardiovascular impact and mortality, which is the reason why we have studied the effects of IH on the structure and function of arteries of aged mice.

6- and 28-month-old females C57Bl6/J mice were placed in IH (5% or 10% O_2) or in normoxia (No, 21% O_2) during 14 days. The mechanics and reactivity of the ascending aorta were studied *ex vivo* by pressure arteriography, while the organization and structure of the arterial wall was investigated by histological technique. Elastin and fibrillin-1 productions by cultured vascular smooth muscle cells (VSMC) was followed by using an ELISA assay, and VSMC calcium signaling was monitored by fluorescence microscopy.

In the aorta of aged animals, our results show that IH induces a substantial increase in inner diameter and stiffness associated to a decreased wall thickness, as compared to the situation in matching animals maintained in normoxia. Consistently, in preliminary studies, the elastics fibers from the aorta of IH mice were more fragmented and, in VSMCs from these animals, synthesis of elastin and fibrillin-1, the major components of the elastic fibers responsible for arterial elasticity, was reduced. However in adult mice, the impact of the IH was low, except for calcium response of VSMCs to phenylephrine, an alpha-adrenergic receptor agonist which is a smooth muscle cell-dependent vasoconstrictor, which was increased in cells from both adult and aged mice which were exposed to IH.

In conclusion, our work shows that IH has more important consequences in aged mice, enhancing the deleterious effects of normal vascular aging. The effects of SAS on the cardiovascular system of the elderly being controversial, it is necessary to continue the investigations in this direction in order to better define and prevent the deleterious effects of SAS in the elderly.

Poster session

Bioactive Elastin-like Recombinamers for Gene Delivery purposes

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The creation of a gene delivery carrier suitable to release the therapeutic gene in the site of action is a challenge in the nanomedicine area. Elastin like recombinamers (ELRs) as recombinant artificial polypeptides whose sequence or a main part of it, mimics the repeated motifs found in natural elastin has been shown as a biocompatible material and a potential source for different applications such as gene delivery. The proteinaceous nature of ELRs allows the addition of cell interaction motifs through genetic engineering techniques. In this study, ELRs were molecularly designed in which cell penetratin peptides with positive charge at neutral pH and fusogenic peptides were incorporated in their sequence with the objective to obtain good transfection efficiency in cells. The polymeric constructions were produced, purificated and characterized. The physical studies of the polymer-pDNA complexes such as particle size, z-potential, stability, complexation capability were accomplished. Their biocompatibility was also tested in terms of blood aggregation and cytotoxicity as well as the transfection

efficiency in C6 glioma rat cell line. The results showed good physical features and biocompatibility regarding to the cell transfection requirements. Concerning to the transfection studies, tested through fluorescence microscopy, polymer-DNA complexes showed internal localization in the cell. The distribution of these nanocomplexes, was found either in the cytoplasmic space or inside of the nucleus.

Rheological synergism between elastin-like based polymers and mucin for possible mucoadhesion devices.

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Adhesion can be defined as the process in which to surfaces interact through interfacial forces, resulting in a more cohesive system. The term bio-adhesion is referred to a specific type of adhesion in which one of the surfaces is related to a biological system. Among these bio-adhesion devices, those who attach to mucus and/or epithelial surfaces are known as mucoadhesives. The concept of mucoadhesion was introduced in the early 80s and is used to define the ability of a polymeric material to interact in a cohesive form with mucous membranes for an extended period of time. There are many theories which describe the process of mucoadhesion, but none of them completely describe the complete process that leads to a mucoadhesive system. More like, each theory describes different steps or types of interaction present in the process of mucoadhesion. Among them, the diffusion theory focuses in the final steps of mucoadhesion, where polymer chains entangle with mucin glycoproteins, leading to a more cohesive system, and thus, facilitating other interactions (electronic, hydrophobic, electrostatic) thanks to the increase in the surface exposed between systems. This increase in molecular interactions should lead to rheological synergism, this is, increased rheological properties of the mixture, in comparison to those of single components. We here present a rheological study of two elastin-like based polymers and their interaction with mucin. ELRs were designed to have a double functionality, a diblock structure that leads to the gelation of

the ELR, and a positively charged hydrophilic tail that allows the ELR to interact with the negatively charged sialic acids present in mucin. Both ELRs differ in the positively charged hydrophilic tail that leads to different mechanical properties and interactions with mucin.

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Developing of a fluorescent silk-elastin-like recombinamer for enhanced tracking in tissue engineering applications

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The increasing applications of genetically engineered recombinamers, such as human elastin derived polypeptides, termed elastin-like recombinamers, in the field of biomedicine have led to the developing of many different devices for tissue engineering, for example biocompatible and bioactive hydrogels used as scaffolds that simulate extracellular matrix.

One of the issues regarding these devices is the correct and certain tracking of the material when implanted or introduced in an organism. For the purpose of addressing this problem, a silk-elastin-like recombinamer (SELR) fused to enhanced green fluorescent protein (EGFP) by recombinant DNA technology has been developed. This recombinamer is able to form nanoparticles at low concentrations and physically cross-linked hydrogels at higher ones, increasing the potential applications of this material.

Finally, when a little amount of the SELR-EGFP is included in the recombinant matrix, it can be tracked through the organism with different *in vivo* imaging instruments by 2-photon excitation and a fluorescence detector, hence succeeding in the determination of the migration and/or degradation of the scaffold through space and time.

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Effect of self-assembled elastin-like recombinamers on the morphologies of calcium phosphate

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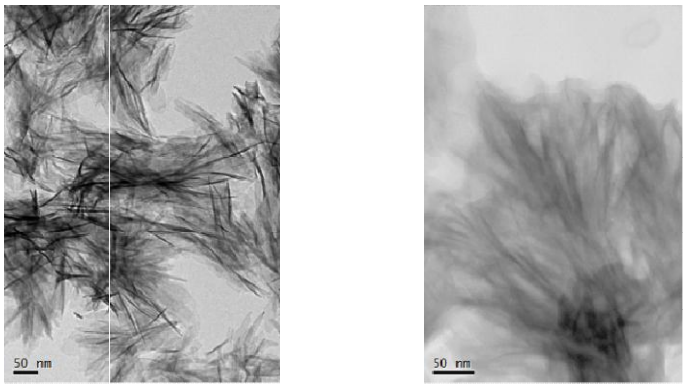
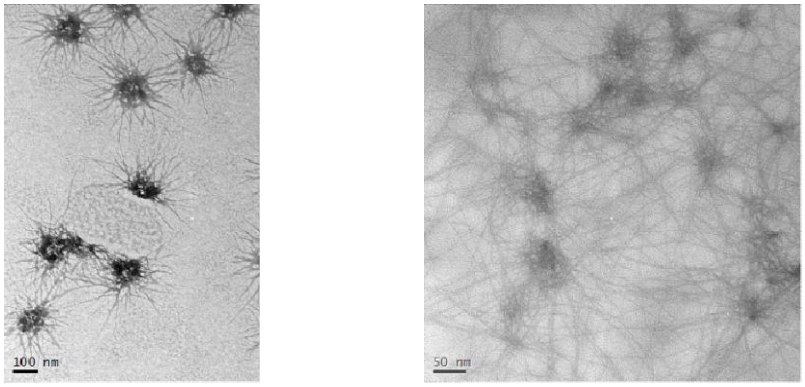
Different calcium phosphate morphologies have been induced in the presence of elastin-like recombinamers (ELRs) that are recombined with the salivary statherin protein fragment, (SN_A15). The type of these morphologies depends on the ELR amphiphilic properties, distribution and number of the SN_A15 fragment attached to the ELR. The SN_A15 fragment recombined at the epitope of an ELR, which have carboxyl groups, induce a feathers-like structure. The increase of SN_A15 fragment number to this ELR induces a star-like or neuron-like structure. This morphology also can be promoted by distributing the SN_A15 fragment along an elastin-like recombinamer that have amine functional groups. The formation mechanism of these different morphologies of calcium phosphate is explained by the Hofmiester effect.



Feathers-like structure of ELR-calcium phosphate



Star or neuron-like structure of ELR-calcium phosphate

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