



XXV Latin Meeting on Vascular Research LIAC 2009

Latinorum Investigatorum de Arteriis Colloquium

SEPTEMBER 2nd – 5th 2009

*Palazzo Gattini Hotel
MATERA, ITALY*



***Commemorating
Prof. A. M. Tamburro***

Potenza, May 2009

Dear Participant

On behalf of LIAC, it is my pleasure to welcome you to the XXV LIAC congress in Matera. LIAC is a working group devoted to the improvement of the quality of basic and applied research in all areas of vascular biology. Our aim is to bring together the scientists of different origins from the entire Latin world in order to increase the exchange and the communication among chemists, biophysics morphologist, molecular biologists, pathologists and physicians with the aim to promote joint and coordinate projects. I am sure the meeting will be very successful because of the important contribution of all of you.

I hope you will have an excellent stay in Matera and a very fruitful congress for all of you.

A. M. Tamburro
The LIAC President

Potenza, August 2009

Dear Colleagues and Friends,

Prof. Antonio M. Tamburro started to make plans for this congress more than 1 year ago, and considered it the finest way to celebrate his 70th birthday. Sadly, we decided to continue the commitment of the LIAC congress, considering it the best tribute to Prof. Antonio M. Tamburro.

We hope you will enjoy your stay in Matera.

Chairpersons of the LIAC 2009
B. Bochicchio & A. Pepe

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We acknowledge contributions of :



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Programme

Wednesday, 2

16.00-18.00 Registration

18.00-18.15 Special opening session in honour of Prof. Antonio M. Tamburro by Jacques Bonnet, LIAC vice-president.

Science and philosophy lecture

18.15-19.15:

Telmo Pievani: "The theory of evolution: the rediscovery of Darwinian pluralism"

19.15-20.00 Cocktail

Thursday, 3

Special session: tribute to Prof. Antonio Mario Tamburro

9.00 – 10.00

- Salutations of Authorities
 - Jacques Bonnet, vice-president of LIAC
 - Manuel Dauchez: A never-ending love story with Elastin
 - Le Voci di Sally, Students and Researchers of University of Basilicata, Italy
- "A tribute concert to Prof. Antonio Mario Tamburro"*

10.30 – 11.00 Coffee break

Molecular and supramolecular structure

Chairs: C. Lacabanne / A.Pepe

11.00-11.30

L1 - Vladimir Uversky, Indiana University, USA

"Unfoldome and Unfoldomics: Introducing Intrinsically Disordered Proteins"

11.30-12.00

L2 - Brigida Bochicchio, University of Basilicata, Italy

"Elastin-derived amyloidogenesis"

12.00-12.30

L3 - Valérie Samouillan, University of Paul Sabatier-Toulouse, France

"Macromolecular dynamics for the analysis of hierarchical organization in elastin-based tissues"

12.30-13.00

L4 - Anna Maria Salvi, University of Basilicata, Italy

"Evidence by surface studies of supramolecular organization induced by the aminoacid specificity of elastin-like sequences in water"

13.00 – 15.00 Lunch

Biomaterials

Chairs: P. Charpiot / M. Spina

15.00 – 15.30

L5 - Giovanni Marletta, University of Catania, Italy

“Surface engineering for bio-active surfaces: managing from nanometric to mesoscopic level”

15.30 – 16.00

L6 - Monica Dettin, University of Padova, Italy

“Bioactive surfaces and biomimetic materials through synthetic peptides grafting”

16.00 – 16.30

L7 - Freddy Gaboriau , University of Toulouse, France

“Plasma processed Carbon-based materials for stent coating”

16.30 – 17.00

Coffee break

17.00 – 17.30

L8 - Julia Bujan, University of Alcalà de Henares, Spain

“Bioactive polymers systems back up the interface of vascular prosthesis”

17.30 – 18.00

L9 - Véronique Larreta Garde, University of Cergy-Pontoise, France

“Use of enzymes to generate smart, programmed and dynamic biomaterials”

18.00 – 18.15

C1- Valentina Santopietro, University of Basilicata, Italy

“ Preliminary structural studies on chimeric protein obtained by Recombinant DNA technologies”

18.15-18.30

C2- Marina Lorusso, University of Basilicata, Italy

“Cross-linked Exon 28 Coded Polypeptide: a Minielastin?”

18.30-19.00

Business LIAC Meeting

Friday, 4

Matrix and Cellular signaling

Chairs:F. Ortolani / L. Martiny

9.00 – 9.30

L10 - Marina Glukhova, CNRS-Institut Curie, Centre de Recherche, Paris, France

“Cell- extracellular matrix interactions in mammary development”

9.30 – 10.00

L11 - Sylvie Ricard-Blum, University of Lyon, France

“The interaction network of endostatin, a fragment of collagen XVIII: from molecular interactions to the control of angiogenesis”.

10.00 – 10.30

L12 - Laurent Duca, University of Reims Champagne-Ardenne, France

“Elastin binding protein-derived signaling: involvement in cardioprotection”

10.30 – 11.00 Coffee break

11.00 – 11.30

L13 - Faustino Bisaccia, University of Basilicata, Italy

"ABCC6 gene and Pseudoxanthoma Elasticum: which link?"

11.30 – 12.00

L14 - Paolo Romagnoli, University of Florence, Italy

"Rosiglitazone modulates the cellular and molecular response to experimental arterial injury"

12.00 – 12.30

L15 - Antonio J. Lepedda, University of Sassari, Italy

"Protein sulfhydryl-group oxidation in stable and unstable human carotid plaques "

12.30 – 13.00

L16 - Natalio García-Honduvilla, University of Alcalà de Henares

"Proadrenomedullin N-terminal peptide and its angiogenic role in repairing ischaemic cutaneous defects"

13.00 – 15.00

Lunch

Vascular walls

Chairs: J. Bujan/ M. Dauchez

15.00 – 15.30

L17 - Robert Mecham, Washington University, USA

"Elastin and the evolution of the vertebrate cardiovascular system "

15.30 – 16.00

L18 - Michel Spina, University of Padova, Italy

"The aortic and the pulmonary heart valve conduits: distribution of structural components and impact of detergent-based cell removal"

16.00 – 16.30

L19 - Alain-Pierre Gadeau, University of Bordeaux Victor Ségalen, France

"Sonic Hedgehog-mediated angiogenesis in the mouse ischemic limb requires osteopontin"

16H30 – 17.00

Coffee break

17.00-17.30

L20 - Ivonne Pasquali-Ronchetti, University of Modena and Reggio Emilia, Italy

"Elastic fiber calcification: new approaches to an old problem."

17.30 – 18.00

L21 - Gilles Faury, Université Joseph Fourier, France

"Fibrillin-1 and Marfan syndrome: fibrillin-1 signalling and lessons from the mouse model Fbn-1 +/mgDelta."

18.00 – 18.15

C3 - Cinthia G. Trejo, University of Alcalà de Henares, Spain

"Expression of progesterone, estrogen and androgen receptors in chronic venous insufficiency"

18.15 – 18.30

C4 - Barbara Perez-Köhler, University of Alcalà de Henares, Spain

"Individual counterbalance between endothelial and smooth muscle cells after hypoxia shock"

20.00 Gala Dinner

Saturday, 5

Vascular disorders

Chairs: M. Formato/ G. Faury

9.30 – 10.00

L22 - Philippe Charpiot; University of Marseille, France

"Early impact of intrauterine growth restriction and premature birth on arterial wall structure"

10.00- 10.30

L23 - Fulvia Ortolani, University of Udine, Italy

"Cardiac valve calcification: new insights from ultrastructural and histochemical analysis, a lesson from "in vivo" models and new perspectives from "in vitro" models"

10.30 – 11.00 Coffee break

11.00 – 11.30

L24- Maurizio Cotrufo & Alessandro Della Corte, Second University of Naples, Italy

"Ascending Aorta Dilatation with Congenital Bicuspid Aortic Valve: from bedside evidence to biomechanics and biomolecular Insights".

11.30: **Concluding remarks: Jacques Bonnet, Vice-president of LIAC**

13.00 – 15.00 Lunch

15.00-17.30

Visit to Sassi district
(Unesco Heritage site)

Abstracts

Science and philosophy lecture

La teoria dell'evoluzione oggi: la riscoperta del pluralismo darwiniano

The theory of evolution: the rediscovery of Darwinian pluralism

Telmo Pievani

Università degli studi di Milano Bicocca, email: telmo.pievani@unimib.it

La teoria dell'evoluzione è rappresentata oggi da un programma di ricerca composito, dotato di un "nucleo" centrale neodarwiniano esteso (mutazione, selezione, deriva e processi macroevolutivi) e di una "cintura" di assunzioni ausiliarie in via di affinamento, riguardanti i ritmi del cambiamento e della speciazione, i livelli di selezione, le sorgenti di variazione e le modalità di ereditarietà, le relazioni fra evoluzione e sviluppo. Gli avanzamenti della genomica evuzionistica, della biologia evolutiva dello sviluppo e della paleoantropologia possono essere efficacemente inquadrati in questa cornice. Ne risulta che è infondato parlare di più teorie dell'evoluzione o di un superamento dell'impianto esplicativo neodarwiniano. Si prefigura piuttosto la corroborazione di quella visione del processo evuzionistico che Stephen J. Gould aveva definito in modo suggestivo come "darwinismo esteso" o "pluralismo darwiniano". Questo approccio alla teoria dell'evoluzione può essere riscontrato nell'opera del fondatore stesso, Charles Darwin, e in particolare nei Taccuini della Trasmutazione (1836-1844) dove per la prima volta le sue idee presero forma. Si prenderà spunto proprio dai Taccuini inediti per evidenziare alcuni aspetti dell'originario pluralismo darwiniano.

La conferenza è tenuta in onore e in ricordo del Magnifico Rettore, professor Antonio Tamburro.

We would like to present here a supposed extension of the neo-Darwinian structure of the theory of evolution. This structure is well represented as a progressive "Scientific Research Program", according to Imre Lakatos' definition. We currently see an extended neo-Darwinian core (variation, natural selection, genetic drift, migration and macro-evolutionary effects), surrounded by a protective belt of auxiliary assumptions in progress, concerning multiple patterns about rhythms of speciation, levels of selection, relationships between evolution and development, and factors of adaptive biological change. The new branching pattern of hominid evolution and "Evo-Devo" are two good case-studies for this hypothesis in philosophy of biology. It is interesting that this pluralistic approach to the theory of evolution could be found in the work of the founder himself, Charles Darwin, and particularly in his "Transmutation Notebooks" (1836-1844), where his ideas took form for the first time.

The lecture is given in honour of Professor Tony Tamburro.

Telmo Pievani

è professore associato di Filosofia della Scienza presso l'Università degli studi di Milano Bicocca, dove è vice-direttore del Dipartimento di Scienze Umane per la Formazione. E' segretario del Consiglio Scientifico del Festival della Scienza di Genova e co-Direttore Scientifico del Festival delle Scienze di Roma. E' autore di 86 pubblicazioni, fra le quali: *Homo sapiens e altre catastrofi* (Meltemi, Roma, 2002); *Introduzione alla filosofia della biologia* (Laterza, Roma-Bari, 2005); *La teoria dell'evoluzione* (Il Mulino, Bologna, 2006); *Creazione senza Dio* (Einaudi, Torino, 2006, finalista Premio Galileo e Premio Enrico Fermi); *In difesa di Darwin* (Bompiani, Milano, 2007); *Nati per credere* (Codice Edizioni, Torino, 2008, con V. Girotto e G. Vallortigara). Alcuni di questi volumi sono in corso di traduzione in lingue straniere, fra le quali inglese e portoghese. Socio corrispondente dell'Istituto Veneto di Scienze, Lettere e Arti per la classe di Scienze, è coordinatore nazionale di un Progetto di ricerca di interesse nazionale (PRIN 2007) sul comportamento adattativo dei sistemi biologici. E' membro dell'editorial board delle riviste scientifiche internazionali *Evolutionary Biology* e *Evolution: Education and Outreach*. E' direttore di *Pikaia*, il portale italiano dell'evoluzione, e coordinatore scientifico del Darwin Day di Milano. Insieme a Niles Eldredge, è direttore scientifico del progetto enciclopedico "Il futuro del pianeta" di UTET Grandi Opere. Insieme a Niles Eldredge e Ian Tattersall, è il curatore scientifico dell'edizione italiana della mostra internazionale "Darwin.1809-2009" (Roma-Milano 2009). Collabora regolarmente con *Il Corriere della Sera* e con le riviste *Le Scienze*, *Micromega* e *L'Indice dei Libri*.

L1-Unfoldome and Unfoldomics: Introducing Intrinsically Disordered Proteins

Vladimir Uversky

Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Health Information and Translational Sciences Building, 410 W. 10th Street, HS5009, Indianapolis, Indiana 46202, USA

Intrinsically disordered proteins (IDPs) lack stable tertiary and/or secondary structure under physiological conditions *in vitro*. They are highly abundant in nature, as ~25-30% of eukaryotic proteins are mostly disordered, and >50% of eukaryotic proteins and > 70% of signaling proteins have long ID regions. Often, these IDPs are involved in regulation, signaling and control pathways, where binding to multiple partners and high-specificity/low-affinity interactions play a crucial role. It is suggested that functions of IDPs may arise from the specific disorder form, from inter-conversion of disordered forms, or from transitions between disordered and ordered conformations. The choice between these conformations is determined by the peculiarities of the protein environment, and many IDPs possess an exceptional ability to fold in a template-dependent manner. IDPs are highly abundant among hub proteins. They are associated with alternative splicing. This association helps proteins to avoid folding difficulties and provides a novel mechanism for developing tissue-specific protein interaction networks. Numerous IDPs are associated with such devastating maladies as cancer, cardiovascular disease (CVD), amyloidoses, neurodegenerative diseases, and diabetes. Novel strategies for drug discovery are based on these proteins.

L2-Elastin-derived amyloidogenesis

Brigida Bochicchio

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Amyloid fibrils are associated to a large number of diseases such as Alzheimer's dementia (AD) and others. Many evidences link AD with vascular diseases and only few data connect amyloids and atherosclerosis and aging via deposits in the aortic intima. Recent results demonstrate that also some elastin domains are able to give amyloid fibers *in vitro*^{1,2,3}. The motif considered to be responsible for amyloid fibrillogenesis is (XGGZG)_n X, Z = Val and/or Leu. It is tempting to speculate that in vascular diseases, under high lipids concentration, some N- and C-terminal sequences, released from elastin by enhanced proteolytic activity, could aggregate because of mutated microenvironment to form amyloids (whose formation is also favoured by lipids) that constitutes the "elastotic material" described in several reports. In order to demonstrate this hypothesis we synthesized and performed physico-chemical studies on exon30-coded derived polypeptide sequences, resulting from cleavage by enzyme such as MMP-12 in the human skin elastin⁴, in order to investigate their amyloidogenic behavior. The results demonstrate that the longest polypeptide sequence, among those synthesized, is able to give rise to amyloid-like fibers *in vitro*.

References

1. A. M. TAMBURRO et al. J. Biol. Chem. 280, 2682-2690 (2005).
2. B. BOCHICCHIO et al. . Biomacromolecules, 8, 3478-86, (2007).
3. A. OSTUNI, et al. Biophys. J., 93, 1-12 (2007).
4. S. TADDESE et al. , Matrix Biol., 27, 420-8 (2008).

L3-Macromolecular dynamics for the analysis of hierarchical organization in elastin-based tissues.

V. Samouillan, J. Dandurand, D. Tintar and C.Lacabanne

Institut Carnot, CIRIMAT UMR 5085, Laboratoire de Physique des Polymères, Université Paul Sabatier, Toulouse, France. vsamou@cict.fr

An important goal in elastin research is the revealing of structure-function relationship, peculiarly to explain the mechanism of elasticity. Due to the extreme insolubility of elastin and so far the difficulty to access to its structure via classical techniques of proteins characterization, that require soluble proteins, different ways have been explored for several years.

In this talk we review the different physical characterizations we performed in our laboratory, in collaboration with chemist and biochemist teams, by the use of various approaches to get insight into the molecular mobility of elastin. All the presented results deal with the adaptation of thermal, mechanical and dielectric techniques, widely used for the analysis of synthetic macromolecules, to the characterization of biomacromolecules.

One way consists in the characterization of peculiar sequences of elastin synthesized by Tamburro's group¹. The study of single domains of tropoelastin to get information on the entire protein has been used and validated by previous results. As a matter of fact, this so called "reductionist approach" has been repeatedly demonstrated to be able to give significant insights on the elastin structure. This so-called "reductionist approach" allows to evidence the distinct answer of hydrophobic and alanine-rich regions of elastin, correlating the conformation to the physical behaviour. Another way is the study of soluble derivatives of elastin. In order to extract the influence of the architecture (i.e. the three-dimensional network) on the chain dynamics of elastin, the insoluble products of elastin obtained by enzymatic digestion, as well as the soluble, uncross-linked derivative of elastin (kappa-elastin) are analysed by these different different techniques². The last way mentioned in our talk is the investigation of insoluble elastin itself and elastic fibers, since our techniques of characterization are well-suited to the analysis of insoluble materials. In this case the combined use of our techniques is presented, allowing us to obtain an identity card for elastin and elastic tissues. The collected database is then used to evaluate the influence of pathological and pharmacological factors on the physical structure and molecular mobility of elastin³, and to contribute to the conception of bioprostheses⁴.

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2. Samouillan, V., Dandurand, J., Lacabanne, C., Hornebeck, W. *Biomacromolecules*. 2002, 3(3), 531.
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4. Samouillan, V., Lamure, A., Maurel, E., Dandurand, J., Lacabanne, C., Spina, M. *Journal of Biomaterials Science : Polymer Edition*. 2000, 11, 583.

L4-Evidence by surface studies of supramolecular organization induced by the aminoacid specificity of elastin-like sequences in water

Pasquale Moscarelli,^a Giuseppina Satriano,^a Anna Maria Salvi,^a Giuseppe Lanza,^b James E. Castle^c and A.M.Tamburro^a

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Elastin-like polypeptides are capable of adopting complex supramolecular structures, showing either a hydrophobic or a hydrophilic surface, depending on both their surrounding environment and the character of any substrate on which they are supported. Knowledge of the preferred organization is important in many situations – ranging from issues of biocompatibility to consideration of their biofunction.

In this work the pentamer LeuGlyGlyValGly was studied as a single monomer and as a polymer, (LeuGlyGlyValGly)_n, and compared with ValGlyGlyLeuGly and ValGlyGlyValGly, all in the form of deposits from pure water suspension on silicon substrates.

These sequences, much repeated in the hydrophobic domain of elastin, are used when suspended in pure deionised water, to represent a very simplified system from which to derive information on the 'in vivo' intermolecular interactions of elastin itself.

Combined surface studies performed in identical conditions have clearly shown differences in the water-induced assembly of these sequences due to the simple substitution and/or permutation of a single aminoacid. XPS, FTIR and AFM experiments were compared with spectra computation in order to derive a model for relating the aminoacids specificity with the aggregation pathways.

Acknowledgments: Dr. Fausto Langerame for XPS acquisition, Dr. Neluta Ibris and Dr. Roberta Flamia for AFM images are gratefully acknowledged. Synchrotron spectra were acquired at the Elettra Beamline Centre of Trieste (Italy).

References:

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4. Antonietta Pepe, Brigida Bochicchio & Antonio M. Tamburro *Nanomedicine* (2007), 2(2), 203-218.
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L5-Surface engineering for bio-active surfaces: managing from nanometric to mesoscopic level

Giovanni Marletta

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The use of Low-Energy Ion Beams (LEIB) to construct bioactive surfaces is discussed, with particular reference to the relevance of chemical and/or morphological structuring of polymeric as well as inorganic surfaces. In particular, the surface physico-chemical factors relevant to cell, bacteria and biomolecule adhesion/adsorption onto surfaces will be discussed in connection with the peculiar LEIB-induced modification of polymer structure and properties.

As the cell interaction with surfaces, propaedeutic to complex tissue formation, is basically determined by the organization of peptides and proteins from the Extracellular Matrix (ECM) with such surfaces, the talk will give at first a glance on the LEIB-induced surface modification of protein conformation and organization at the nanoscale (see for instance fig.1).

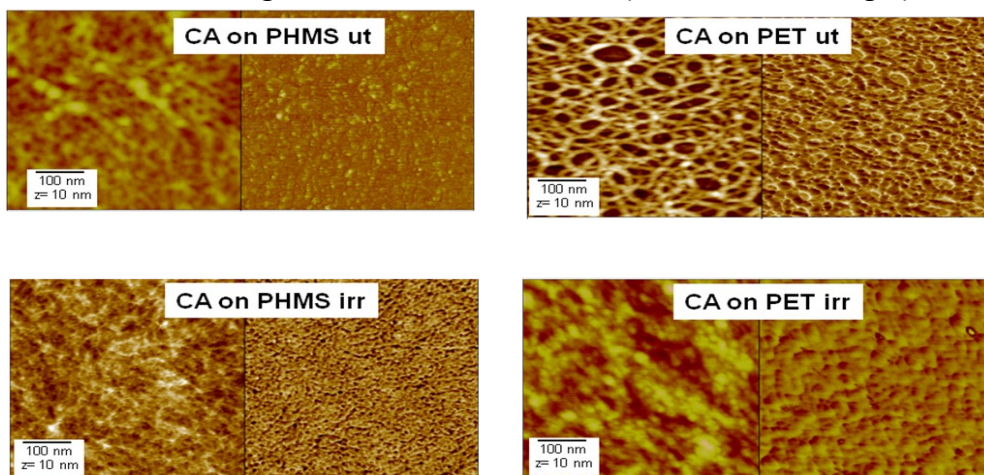


Fig.1 – Different organization at nanoscale of Collagen type I on polymer surfaces before (ut) and after irradiation (irr) with Low-Energy Ion Beams.

The adsorption/organization processes of proteins will be shortly discussed in view of the chemical and physical structure of the surfaces obtained by using Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), Small Spot X-Ray Photoelectron Spectroscopy (XPS), Near Field Microscopies (NFM), and Surface Free Energies measurements (SFE).

The focus will then be moved to the analysis of the cell (or bacteria) behavior on LEIB-modified polymeric surfaces. Thus, the enhanced adhesion and spreading response of fibroblasts, endothelial cells, pericytes and osteoblasts to irradiated surfaces will be phenomenologically correlated to the formation of specific functional groups at surface, to the surface charge state, to the surface free energy parameters as well as to topography, including the problem of the relevance of nanometric scale modifications.

In particular, it will be shown that LEIB apparently is able to induce very peculiar surface modifications, determining highly specific cell response with respect to other available surface engineering methods, like for instance plasmas or adhesive peptide grafting. Examples will also be shown of the endocellular “structuring” depending on the irradiation conditions.

Finally, it will be shown that the adsorption behavior of proteins as well as the global response of cellular systems may be globally understood in terms of the relative weight of the various components of the surface free energy, nanometric-scale structure and electrical properties of the irradiation-modified polymers.

L6-Bioactive surfaces and biomimetic materials through synthetic peptides

grafting

Monica Dettin

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Biomimetic surface design consists in “writing a message” on the surface of a biomaterial just using the biochemical language of the target cells. In the present contribution we will discuss the advantages of synthetic peptides rather than native proteins as biologically active agents; furthermore, we will present different approaches for the delivery of bioactive molecules at tissue-implant interface¹⁻⁴. Our experience in the field of peptide grafting to Ti surfaces will be also presented⁴: innovative methods for peptide covalent immobilization to Ti surface, physical-chemical characterization of biomimetic surfaces, and h-osteoblast adhesion data concerning both cell number and cell adhesion strength quantification will be shown⁵. The results of Total Internal Reflection Fluorescence (TIRF) morphological characterization of h-osteoblasts seeded on peptide-grafted glass surfaces will be also considered: interestingly, pre-treatment of cells with anti-integrin antibodies or with glycosidases gives evidence of the osteoblast adhesion mechanism stimulated by peptides. The experience of our group as to the synthesis of self-assembling peptides⁶ for injectable scaffold for cell recruitment and survival, will be briefly reported: in particular, the results of h-osteoblast adhesion on electrospun hybrid scaffolds composed by biopolymers and a bioresorbable polymer (polyethylene oxide, PEO) will be discussed. A final example of biomimetic modification will concern the covalent cross-linking of biological heart valve collagen through photo-probe labelled peptides or sequences carrying oxyamine groups for increasing the stability and the biomechanical properties of biological prostheses.

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L7-Plasma processed Carbon-based materials for stent coating

Freddy Gaboriau^{1,2,*}, Richard Clergereaux^{1,2}, Muriel Laffargue³

¹ Université de Toulouse; UPS, INPT; LAPLACE (Laboratoire Plasma et Conversion d'Energie); 118 route de Narbonne, 31062 Toulouse cedex 9, France. ² CNRS; LAPLACE; 31062 Toulouse, France.

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To prevent restenosis, different treatments are in development: (i) to limit the proliferation and the migration of CMLs by chemotherapy, (ii) to increase the re-endothelialisation on stent by activating endothelium reconstruction (chemotherapy) or by modifying the stent surface (biocompatibility). Carbon materials have a real interest for a long time¹ as they are known as biocompatible, chemically inert, non corrosive and mechanically compatible with prostheses coatings^{2,3}. Indeed, in the literature, a specific affinity of carbon layer structure on biological media appears: DNA stripping from vinyl groups⁴, polystyrene Petri dishes⁵ are different examples that show a real impact of the carbon structure on biological media.

Among the thin film deposition processes of carbon-based materials, plasma enhanced chemical vapor deposition is an interesting route as it takes place at low temperature (avoiding any effects of heating) and as it is well controlled by the plasma parameters. Schematically, several mechanisms can take place in a plasma deposition process:

- radicals and ions are produced by collision between electrons and precursor molecules. These species drift to the surfaces inducing a thin film deposition. Depending on the plasma parameters, it is possible to form graphite-like, diamond-like or polymer-like materials.
- negative ions can also be produced in the discharge. These species, repulsed from the walls, are confined in the plasma where they can recombine to form graphite-like powders. When encapsulated in the thin film matrix, it is possible to form nanocomposite thin films.

In a recent project⁶ in-vitro adhesion and proliferation of Human Vascular Endothelial Cells (HUVEC) have been studied on plasma deposited carbon-based thin films. These preliminary results, compared to a positive and negative control, show adhesion and proliferation of the same order or overlap the positive control for some specific conditions. From the comparison between the cell culture and the physico-chemical properties of thin film, several correlations can be given: hydrogen concentration on the film surface limits cell adhesion and proliferation, sp² hybridized nanoparticles strongly improve both cell adhesion and proliferation, cell adhesion and proliferation are only linked to the physical-chemical structure of the material and not to the film roughness.

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L8-Bioactive polymers systems back up the interface of vascular prosthesis

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Biomaterials are currently the most common substitutes for human tissue. In spite of new technologies let us to prepare punctual and personal biomaterials, vascular substitutes continue being one difficult option for biomaterials implantation due to the blood interface.

The need for improving the patency of arterial substitutes in distal territories, keeps its medical attention. Only 40% of the small arterial grafts are patency after 7 years postimplantation. Thrombosis and restenosis are the mainspring of the grafts failure.

To avoid the initial thrombosis and restenosis of the arterial grafts, we design some polymer system based on Hidroxyethyl metacrylate (HEMA), containing antithrombogenic sustancias (acetil salicilic acid and triflusal derivatives) and one nanoparticled system based in lactic acid/glycolic acid (PLGA) copolymer containing low molecular weight heparin (LMWH).

Polymeric hydrogels (HEMA) with Acetyl salicilic acid and Triflusal derivatives were use to coat the inner surface of vascular expanded polytetrafluoroethylene, and were tested in “in vitro” and “in vivo” experiments, showing an important effect for inhibiting platelet adhesion in the first moments after blood flow restoration. In vivo, after two months of carotid grafts implantation they, showed an effective 30% increase of patency respect the vascular PTFE grafts used as control group.

Restenosis is a complication for the small vessels grafts. Heparin is well known as inhibitor of smooth muscle cell proliferation. The use of a polymeric system for controlled release of LMWH was tested (PLGA nanoparticles containing 10% Bemiparin) as potential modulator of intimal hyperplasia. However in a surprising way we found that this LMWH essayed showed a significant increase for the smooth muscle cell proliferation in vitro. In this case LMWH present a novel and different effect on smooth muscle cell that needs to be studied in detail.

L9-Use of enzymes to generate smart, programmed and dynamic biomaterials

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In certain biological processes, enzymes catalyze subtle modifications at the molecular scale which induce large variations at the supramolecular level, including phase transitions¹. Several catalysts may be used *in vitro* to generate biogels and/or modify their overall physicochemical properties. Gelatin gel properties are dependent on the equilibrium between antagonistic enzymes² : transglutaminase catalyzes intermolecular protein cross-linking and acts as a reverse protease in biogel formation.

Using an experimental system consisting of gelatin, transglutaminase and collagenase, ephemeral gels named "Enzgels" were obtained *in vitro*³; they are programmed to dissolve after a determined time, without any change in temperature or medium composition, and constitute a completely new type of material^{4, 5}. The diffusion of various components included in these gels may be controlled through the mastered kinetics of biogel degradation.

The development of enzyme-catalyzed gels considerably enlarges the range of mechanical characteristics of biomaterials, offering possible applications in the field of tissue engineering or therapeutic release.

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