2nd INTERNATIONAL CONFERENCE ON MATRIX VESICLES : FROM BIOCHEMISTRY TO CLINIC

ICMV 2019

June 14th 2019

UNIVERSITE LYON 1, ICBMS UMR 5246 CNRS,
BATIMENT LEDERER

GRANDLYON
la métropole

UNIVERSITÉ DE LYON

Université Claude Bernard Lyon 1

UFR Biosciences
Université Claude Bernard Lyon 1

INSTITUT DE CHIMIE ET BIOCHIMIE MOLÉCULAIRES ET SUPRAMOLÉCULAIRES
icbms UMR 5246

fisher scientific
by Thermo Fisher Scientific

A SPECIAL THANK TO OUR SPONSORS

On behalf of the scientific council and of the organizing committee, we wish to thank Grand Lyon La métropole, Fisher scientific, Université Claude Bernard Lyon 1, UFR Biosciences, ICBMS UMR CNRS 53246 for their financial support to the ICMV-2019.

We are grateful to our colleagues José Luis Millán and Dobrawa Napierala from United States, Maytê Bolean, Pietro Ciancaglini and Ana Paula Ramos from Brazil, Agnieszka Strzelecka-Kiliszek from Poland and Massimo Bottini, Italy for their participation in this conference, sharing their expertise with us.

We wish to thank ICBMS for the conference room, building Web site and managerial activity.

Second International Conference on Matrix Vesicles: From Biochemistry to Clinic, June 14th 2019, Villeurbanne

SYNOPSIS

Matrix vesicles are a specific class of extracellular vesicles with a diameter between 100 and 300 nm. Under physiological conditions, they are blebbed from hypertrophic chondrocytes during endochondral ossification, from osteoblasts during intramembranous ossification and from odontoblasts during mantle dentin formation. Matrix vesicles, by accumulating calcium and phosphate, can initiate the first step of nucleation of crystals leading to apatite. The symposium is focused on answering questions such as: How matrix vesicles are released by mineralizing cells? How calcium and phosphate accumulate in matrix vesicles? What are the biochemical and biophysical properties of matrix vesicles? To what extend do they affect ectopic calcification? What clinical applications could be derived from a better knowledge of the functions of matrix vesicles? How proteoliposomes that mimick matrix vesicles could be used in nanomedicine?

TOPICS

- Matrix vesicles formed during physiological and pathological conditions
- Functions of matrix vesicles
- Biogenesis of matrix vesicles
- Methods to analyze the morphology and biochemical and biophysical properties of matrix vesicles
- Determination of calcium and phosphorus inside matrix vesicles
- Lipidomics and phospholipase activity in matrix vesicles
- Proteoliposomes mimicking matrix vesicles

WEB SITE

https://matrix-vesicles.sciencesconf.org

FIRST INTERNATIONAL CONFERENCE ON MATRIX VESICLES : FROM BIOCHEMISTRY TO CLINIC

29th May 2018, Università degli Studi di Roma Tor Vergata, Roma, Italy

2nd INTERNATIONAL CONFERENCE ON MATRIX VESICLES
Villeurbanne, FRANCE
Friday 14th of June 2019

SCIENTIFIC COUNCIL

CHAIR

Professor José Luis Millan	Sanford Burnham Prebys Medical Discovery Institute, La Jolla, USA

MEMBERS

Professor Massimo Bottini	University of Rome Tor Vergata Rome, Italy
Professor René Buchet	ICBMS UMR 5246 - Université Lyon 1 - CNRS - INSA Lyon - CPE Lyon, Villeurbanne France
Professor Pietro Ciancaglini	University of São Paulo Ribeirão Preto, Brazil
Professor David Magne	ICBMS UMR 5246 - Université Lyon 1 - CNRS - INSA Lyon - CPE Lyon, Villeurbanne France
Professor Dobrawa Napierala	University of Pittsburgh School of Dental Medicine - McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh PA , USA.
Professor Slawomir Pikula	Nencki Institute of Experimental Biology Polish Academy of Science, Warsaw, Poland

LOCAL COMITTEE UNIVERSITY LYON 1

Professor René Buchet

Professor David Magne

Associate Profesor Saida Mebarek

2nd INTERNATIONAL CONFERENCE ON MATRIX VESICLES : FROM BIOCHEMISTRY TO CLINIC

PROGRAMM

8H-8H30 Registration and coffee

8H30-8H40 Introduction

Physiology, pathology and biogenesis of matrix vesicles
Moderator: Professor David Magne

8H40-9H20 Professor José Luis Millán
The function of matrix vesicles in physiological and pathological mineralization
Sanford Burnham Prebys Medical Discovery Institute, La Jolla, USA.

9H20-10H Associate Professor Dobrawa Napierala
Molecular Regulation of Matrix Vesicles Biogenesis.
University of Pittsburgh School of Dental Medicine - McGowan
Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh PA, USA.

10H-10H30 : Coffee Break

Methods of investigations of matrix vesicles
Moderator: Professor José Luis Millán

10H30-11H10 Associate Professor Massimo Bottini
Investigation of the biophysical properties of matrix vesicles by quantitative techniques.
University of Rome Tor Vergata Rome, Italy.

11H10-11H50 Associate Professor Agnieszka Strzelecka-Kiliszek
Characteristics of matrix vesicles using X-Ray microanalysis.
Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland.

11H50-12H10 Dr. Sylvie Tambutté
Coral biomineralization and vesicles, Centre Scientifique de Monaco, Marine Biologie Department, Monaco

12H10-14H : Lunch and Poster session

Lipidomics and models of matrix vesicles
Moderator: Professor René Buchet

14H00-14H40 Professor Pietro Ciancaglini
Proteoliposomes as a model for matrix vesicles
University of São Paulo Ribeirão Preto, Brazil.

14H40-15H20 Dr. Maytê Bolean
Application of proteoliposomes matrix vesicle mimetics in the biomineralization and its interactions with collagen matrix.
University of São Paulo Ribeirão Preto, Brazil.

15H20-16H Associate Professor Ana Paula Ramos
Is alkaline phosphatase biomimeticaly immobilized on solid surface able to propagate the biomineralization process ?
University of São Paulo Ribeirão Preto, Brazil.

16H-16H40 Associate Professor Saida Mebarek
Lipidomics on Matrix Vesicles
ICBMS UMR 5246 - Université Lyon 1 - CNRS - INSA Lyon - CPE Lyon, Villeurbanne, France.

16H40-17H Conclusion

17H-18H30 Poster sessions

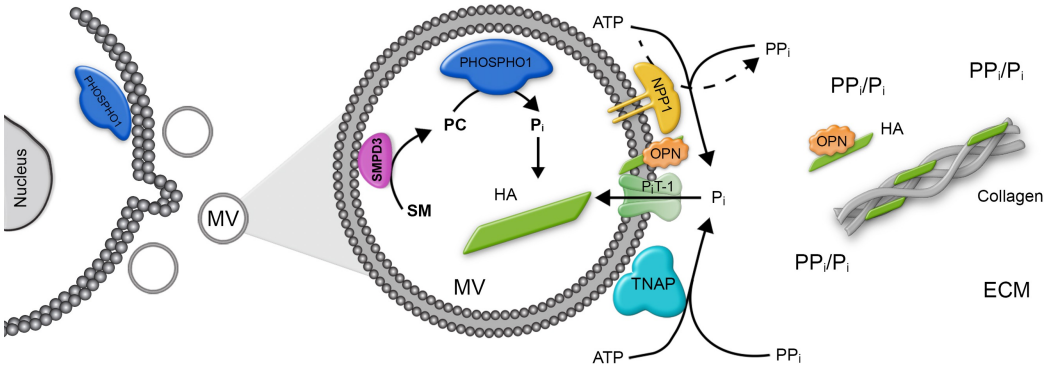
2nd INTERNATIONAL CONFERENCE ON MATRIX VESICLES : FROM BIOCHEMISTRY TO CLINIC

CONFERENCES

The function of matrix vesicles in physiological and pathological mineralization.

José Luis Millán
Sanford Burnham Prebys Medical Discovery Institute, La Jolla, California, USA.
(millan@sbpdiscovery.org)

Proper mineralization of skeletal and dental tissues is fundamental to the fitness and survival of vertebrates. However, the intimate biochemical processes that lead to mineralization of the skeleton have not yet been completely elucidated.
Our current understanding of the biochemical pathways involved in MV-mediated initiation of skeletal, dental and vascular calcification is compatible with the following sequence of events, shown schematically in the figure below: MVs initiate mineral deposition by accumulation of Pi generated intravesicularly by the action of PHOSPHO1 on phosphocholine derived from sphingomyelin by the action of SMPD3, and also via the Pi transporter PiT-1-mediated incorporation of extracellular Pi generated extravesicularly by TNAP and/or NPP1 on ATP. The extravesicular propagation of mineral onto the collagenous matrix is mainly controlled by the pyrophosphatase activity of TNAP that restricts the concentration of this potent mineralization inhibitor to establish a PPi/Pi ratio conducive to controlled calcification. Additionally, osteopontin (OPN), another potent mineralization inhibitor that binds to HA as soon as it is exposed to the extracellular fluid, further restricts the degree of extracellular matrix ECM mineralization. However, how MVs are formed is still unclear and little is known about how apatite crystals formed within MVs propagate onto the collagenous matrix. Our working hypotheses postulate that 1) MV biogenesis is controlled by PHOSPHO1 function and 2) that once blebbed, MVs bind to collagen fibrils through specific molecular interactions mediated in part by TNAP and annexins.Furthermore, 3) once the MV-initiated crystals become exposed to the ECM, binding of OPN to those crystals promotes propagation of mineralization onto the collagenous matrix.



Understanding MV biogenesis and function is critical to being able to develop rational approaches for the prevention of ectopic calcification. Our work has already shown that TNAP inhibitors can prevent soft-tissue calcification in preclinical models of medial vascular calcification. PHOSPHO1 is another druggable target being considered for the prevention of ectopic calcification to be used alone or in combination with TNAP inhibitors. This “dual phosphatase” inhibition strategy might be expected to reduce ectopic calcification by reducing the mineralization ability of

MVs as well as by interfering with MV biogenesis, thus representing a powerful double-hit therapeutic strategy

Molecular Regulation of Matrix Vesicles Biogenesis

[1]Sandeep Chaudhary, [2]Sana Khalid, [2]Mairobys Socorro, [1]Terje Dokland, [3]Massimo Bottini, [4]José Luis Millán, [2,5]**Dobrawa Napierala**

[1]University of Alabama at Birmingham, Birmingham, USA
[2]University of Pittsburgh School of Dental Medicine, Pittsburgh, USA
[3]Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy
[4]Sanford Children's Health Research Center, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, USA
[5]McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, USA (don11@pitt.edu)

Matrix vesicles (MVs) are a specific type of extracellular vesicles with a unique function of supporting the initiation of the mineralization process. Although it has been well documented that MVs are released by many cell types producing mineralizing extracellular matrix, regulation their formation is not well understood. The goal of our studies is to identify extracellular stimuli of MVs release and molecular mechanisms regulating this process. Using cellular models of mineralization, we have demonstrated that committed osteogenic cells rapidly increase formation of MVs, when stimulated with above-physiologic concentrations of extracellular inorganic phosphate (P_i). This P_i-induced release of MVs is dependent on activation of Erk1/2 kinases and is associated with activation of Rac1 and cdc42 small GTPases and reorganization of the actin cytoskeleton. Furthermore, we uncovered that osteogenic cells deficient in mineralization-regulating transcription factor Trps1 have severely impaired MVs biogenesis and mineralization. On the molecular level, this is accompanied by impaired cellular response to P_i, impaired activation of Rac1/cdc42 and downregulated expression of TNAP and PHOSPHO1 phosphatases. In addition, considering that P_i changes gene expression program of osteogenic cells, we have analyzed proteins of MVs released from cells cultured in standard growth medium (containing 0.8mM Pi) with MVs released from cells exposure to 5mM P_i. Mass spectrometry and protein profiling analyses revealed that P_i affects protein composition of MVs, however we did not establish a correlation between P_i-regulated gene expression and P_i-regulated composition of MVs. In summary, our studies suggest that P_i is not only a stimulator of MVs biogenesis, but also a regulator of MVs content. Furthermore, Trps1 transcription factor regulates biogenesis of MVs, at least in part, by regulating the cellular response to P_i and expression of kinases essential for MVs.

Investigation of the biophysical properties of matrix vesicles by quantitative techniques

Justin S. Plaut,[a,b] Agnieszka Strzelecka-Kiliszek,[c] Lukasz Bozycki,[c] Slawomir Pikula,[c] René Buchet,[d] Saida Mebarek,[d] Meriem Chadli,[d] Maytê Bolean,[e] Ana M. S. Simao,[e] Pietro Ciancaglini,[e] Andrea Magrini,[f,g] Nicola Rosato,[g,h] David Magne,[e] Agnès Girard-Egrot,[e] Colin Farquharson,[i] Sadik C. Esener,[a,b] José L. Millan,[j] **Massimo Bottini**[g,h,j]

[a] Cancer Early Detection Advanced Research Center, Knight Cancer Institute, Oregon Health & Science University, Portland, Oregon 97201, USA. [b] Department of Bioengineering, University of California San Diego, La Jolla, California 92093, USA. [c] Laboratory of Biochemistry of Lipids, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 02-093 Warsaw, Poland. [d] Université de Lyon, Université Lyon 1, Institut de Chimie et Biochimie Moléculaires et Supramoléculaires, UMR CNRS 5246, 69 622 Villeurbanne Cedex, France. [e] Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto – USP, Departamento de Química, 14040-901 Ribeirão Preto, Brasil. [f] Department of Biopathology and Imaging Diagnostics, University of Rome Tor Vergata, Rome, Italy. [g] Nanoscience & Nanotechnology & Innovative Instrumentation (NAST) Centre, University of Rome Tor Vergata, Rome, Italy. [h] Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy. [i] Division of Developmental Biology, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Roslin, Midlothian, Edinburgh EH25 9RG, UK. [j] Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037, USA. (mbottini@sbpdiscovery.org)

ABSTRACT

Matrix vesicles (MVs) are a class of extracellular vesicles that form crystalline complexes in their lumen. The ability of MVs to induce the formation of crystalline complexes resides in the presence of a nucleation core (NC) in the lumen of nascent vesicles. Direct evidence of the existence of the NC and its maturation has been provided exclusively by analyses of dried samples. In this work, we provided evidence of the presence of the NC and the changes in its physical properties during mineralization by using peak force quantitative nanomechanical atomic force microscopy (PFQNM-AFM) on hydrated MVs immersed in synthetic cartilage lymph (SCL) without and with 2mM Ca^{2+} as mineralization trigger. In particular, we found that the MVs' elastic modulus significantly increased after incubation in mineralization buffer. Dynamic light scattering showed that the hydrodynamic diameter of hydrated MVs increased significantly, whereas the vesicles' surface charge did not change after incubation with Ca^{2+}. This result validates that crystalline complexes, which are strongly negative, formed within MV lumen. These data were substantiated by transmission electron microscopy energy dispersive X-ray and Fourier transform infrared spectroscopic analyses of dried MVs, which provided evidence that the NC increased in size, crystallinity, and Ca^{2+}/P_i ratio within MVs during the biomineralization process.

Characteristics of matrix vesicles using X-ray microanalysis

Agnieszka Strzelecka-Kiliszek, Lukasz Bozycki, Lilianna Grochocka, Magdalena Komiażyk-Mikulska, Monika Roszkowska, Slawomir Pikuła

Laboratory of Biochemistry of Lipids, Nencki Institute of Experimwental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland (a.strzelecka-kiliszek@nencki.gov.pl)

We used transmission electron microscopy with energy dispersive X-ray microanalysis (TEM-EDX) to compare the composition of minerals in vesicles released by two bone cell lines: hFOB 1.19 and Saos-2 and two vascular smooth muscle cell lines: MOVAS and HCASMC. These cell lines, after treatment with ascorbic acid (AA) and β-glycerophosphate (β-GP), undergo complete osteogenic transdifferentiation from proliferation to mineralization and produce matrix vesicles (MVs) that trigger apatite nucleation and their deposition in the extracellular matrix (ECM). It must be underlined that tissue-nonspecific alkaline phosphatase (TNAP) and collagen are the two proteins necessary for physiological mineralization in bones as well as for vascular calcification during atherosclerosis.

Based on Alizarin Red-S (AR-S) staining and analysis of the composition of minerals in cell lysates using ultraviolet (UV) light or in vesicles using TEM imaging followed by EDX quantitation and ion mapping, we can infer that osteosarcoma Saos-2 and osteoblastic hFOB 1.19 cells reveal distinct mineralization profiles. Saos-2 cells mineralize more efficiently than hFOB 1.19 cells and produce larger mineral deposits that are not visible under UV light. These minerals are similar to hydroxyapatite (HA), however, they have more Ca and F substitutions. We also demonstrate the presence of membrane-bound TNAP, detected by immunofluorescence, that seems to form clusters on the plasma membrane of MOVAS cultured in mineralizing conditions. We observed that TNAP activity and mineralization were increased when MOVAS and HCASMC were cultured in the presence of AA and β-GP. Increased TNAP activity was observed in whole cell lysates, total membrane fractions and, more particularly, in MVs. We have shown that TNAP-enriched MVs released from MOVAS subjected to collagenase treatment, contained more apatite-like mineral than vesicles isolated without collagenase digestion, suggesting a role for collagen in promoting calcification induced by TNAP in atherosclerotic plaques.

The results obtained using X-ray microanalysis allow us to conclude that the process of mineralization differs depending on the cell type. We propose that, at the cellular level, the origin and properties of vesicles predetermine the type of minerals formed.

Coral biomineralization and vesicles

[1]Philippe Ganot, [1]Eric Tambutté, [1]Alexander Venn, [1]Duygu Sevilgen, [1]Natacha Caminiti-Segonds, [1]Nathalie Techer, [1]Denis Allemand, [1]Sylvie Tambutté
[1]Centre Scientifique de Monaco, Marine Biologie Department, 98 000 Monaco
stambutte@centrescientifique.mc

In tropical corals, biomineralization leads to the formation of an exoskeleton made of calcium carbonate (aragonite) and organic matrix molecules including proteins, lipids and polysaccharides. This process is under a biological control in that cells synthesize the skeletal organic matrix molecules as well as they regulate pH, calcium and dissolved inorganic carbon at the site of calcification through ion transporters and enzymes. This favourable physico-chemistry at the site of calcification allows nucleation and crystal growth in the presence of the skeletal organic matrix. Extracellular vesicles have been observed for a long time both inside and between calcifying cells, and more recently, they have been proposed to play a crucial role in coral biomineralization. However, data are in their infancy and further work is absolutely needed to better characterize these vesicles, determine their content and link the current pieces of the puzzle from the cell/vesicles to crystal formation

Proteoliposomes as a model for matrix vesicles

[1]Maytê Bolean, [1]Bruno Z. Favarin, [1]Heitor G. Sebinelli, [1]Ekeveliny A. Veschi, [1]Cláudio R. Ferreira, [2,3]Massimo Bottini, [3]José L. Millán,[1]**Pietro Ciancaglini**
[1]Depto. Química, FFCLRP-USP, Ribeirão Preto, SP, Brasil (pietro@ffclrp.usp.br)

[2]Dept. Experimental Medicine, University of Rome Tor Vergata, Rome, Italy
[3]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA;

During the process of endochondral bone formation, chondrocytes and osteoblasts mineralize their extracellular matrix (ECM) by promoting the synthesis of hydroxyapatite (HA) seed crystals in the sheltered interior of membrane-limited matrix vesicles (MVs). Several lipid and proteins present in the membrane of the MVs mediate the interactions of MVs with the ECM and regulate the initial mineral deposition and posterior propagation. Among the proteins of MV membranes, ion transporters control the availability of phosphate and calcium needed for initial HA deposition. Phosphatases (orphan phosphatase 1, ectonucleotide pyrophosphatase/ phosphodiesterase 1 and tissue-nonspecific alkaline phosphatase) play a crucial role in controlling the inorganic pyrophosphate/inorganic phosphate ratio that allows MV-mediated initiation of mineralization. The lipidic microenvironment can help in the nucleation process of first crystals and also plays a crucial physiological role in the function of MV-associated enzymes and transporters (type III sodium-dependent phosphate transporters, annexins and Na^+,K^+-ATPase). The whole process is mediated and regulated by the action of several molecules and steps, which make the process complex and highly regulated. Liposomes and proteoliposomes, as models of biological membranes, facilitate the understanding of lipid-protein interactions with emphasis on the properties of physicochemical and biochemical processes. We discuss the use of proteoliposomes as multiple protein carrier systems intended to mimic the various functions of MVs during the initiation and propagation of mineral growth in the course of biomineralization. We focus on studies applying biophysical tools to characterize the biomimetic models in order to gain an understanding of the importance of lipid-protein and lipid-lipid interfaces throughout the process. (Financial Supports: FAPESP 2014/11941-3 and 2016/21236-6; CAPES 88887.320304/2019-00; CNPq 306166/2013-5 and 167497/2017-0)

Application of proteoliposomes matrix vesicle mimetics in the biomineralization and its interactions with collagen matrix.

[1]**Maytê Bolean,** [1]Luiz H. da Silva Andrilli, [2]José Luis Millán, [3]Marc F. Hoylaerts and [1]Pietro Ciancaglini

[1]Depto. Química, FFCLRP-USP, Ribeirão Preto, SP, Brasil (maytebolean@usp.br);
[2]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, USA;
[3]University of Leuven, Leuven, Belgium.

Proteoliposomes are useful as mimetics of mineralizing cell-derived matrix vesicles (MVs), known to be responsible for the initiation of endochondral ossification, as they successfully transport Ca^{+2} and possess the ability to hydrolyze phosphosubstrates in the lipid-water interface. During the last decade, these systems have gained prominence as nanotools for biophysical and biochemical studies on lipid–protein interactions as well as for their biotechnological applications. It has been found that TNAP-proteoliposomes have the ability to induce the *in vitro* mineralization even in the absence of mineral nucleators. Besides that, the presence of ordered domains due to the addition of Chol to DPPC or DPPC:SM-proteoliposomes harboring TNAP reduced the ability of the enzyme to induce biomineralization. Thus, the physical properties and the lateral-phase organization of lipids in proteoliposomes are relevant to regulate the apatite propagation mediated by TNAP function during mineralization. Proteoliposomes harboring AnxA5, TNAP or both proteins together could bind to collagen fibers, however AnxA5 binds with the highest affinity (~70%). TNAP-proteoliposomes do poorly bind to collagen matrix (< 20%). The best affinity is for a type II collagen matrix. Besides that, AnxA5-proteoliposome interacts considerably better with collagen matrix when AnxA5 is in the DPPC:DPPS-liposome (around 3 times more) when compared with vesicles composed by neat DPPC. The negatively charged lipid (DPPS) plays a very important role both improving the AnxA5 incorporation into liposomes and it favors the binding of proteoliposomes to the collagen matrix. These findings together highlight the current prospects of this technology for biotechnological applications, including the construction of a multiprotein nanovesicular biomimetic to study the processes of initiation and propagation of skeletal mineralization. (Financial Supports: FAPESP 2014/11941-3 and 2016/21236-6; CAPES 88887.320304/2019-00; CNPq 306166/2013-5 and 167497/2017-0).

Is alkaline phosphatase biomimeticaly immobilized on titanium able to propagate the biomineralization process?

Marcos A. E. Cruz[1], Camila B. Tovani[1], Mayte Bolean[1], Pietro Ciancaglini[1] and **Ana Paula Ramos[1]**

[1]Department of Chemistry, University of São Paulo, São Paulo, SP-Brazil (anapr@ffclrp.usp.br)

Langmuir monolayers (LM) and Langmuir-Blodgett films (LB) containing phospholipids and osteogenic proteins can serve as biomimetic models for biomineralization studies once they act as matrices for controlled precipitation of biominerals. Calcium carbonates, phosphates, and also cation-substituted apatites can be homogeneously deposited on these surfaces in a well-ordered array depending on the type of the phospholipid and the precipitation method. The biomimetic models were formed by the exposure of the LM and LB to solutions that simulate the ionic concentration and pH of the human blood-plasma. Cations (Ca^{2+} and/or Sr^{2+}) were transferred to the films binding to the phospholipids polar-heads. The induction time of precipitation is controlled by the composition of the monolayers, revealing the importance of charges and packing on the mineral formation. It was possible to keep the phophohydrolytic activity of tissue non-specific alkaline phosphatase (ALP) after immobilization on LM and LB. ALP was able to hydrolyze organic-phosphate from ATP, inducing the formation of apatites in a biomimetic way. These set of results attest the reliability of these models for studies of biomineralization. (FAPESP 2017/08892-9)

Lipidomics on matrix vesicles

Dina Abdallah[1,2], Eva Hamade [2], Badran Bassam[2],
Cyril Thouverey [1, 3], Agnieszka Strzelecka-Kiliszek [3],Slawomir Pikula[3] , René Buchet [1]
and **Saida Mebarek [1]**

[1] Université de Lyon, Lyon, F-69361, France Université Lyon 1, Villeurbanne, F-69622, France INSA-Lyon, Villeurbanne, F-69622, France CPE Lyon, Villeurbanne, F-69616, France ICBMS CNRS UMR 5246, Villeurbanne, F-69622, France. (saida.mebarek@univ-lyon1.fr)

[2]Genomic and Health laboratory/PRASE-EDST Campus Rafic Hariri-Hadath-Beirut-Liban, Faculty of Sciences, Lebanese university, Beirut 999095, Lebanon
[3]Laboratory of Biochemistry of Lipids, Nencki Inst. Exp. Biol., 02-093 Warsaw, Poland.

Cells secrete in extracellular medium various types of microparticles of size ranging from 50 nm to 1000 nm, including exosomes, apoptotic bodies and vesicles. Matrix vesicles (MVs) are the particular vesicles released by hypertrophic chondrocytes and osteoblasts to promote bone mineralization. MVs initiate the formation of apatite by accumulating Ca^{2+} and phosphate. Despite a burgeoning interest in extracellular vesicles, little is known about the processes through which these extracellular organelles are generated Three distinct types of microparticles were isolated : exosomes and vesicles ; apoptotic bodies and MVs released from collagenase treatment. In contrast to other microparticles, only MVs were able to initiate mineralization and were enriched in tissue non-specific alkaline phosphatase. The phospholipid composition of the MV and microvilli-like membrane fractions was markedly different from that of the membrane chondrocytes. These MVs and microvilli produced by epiphyseal cartilage cells or by hypertrophic chondrocytes were enriched in cholesterol, phosphatidylserine, and sphingomyelin as in the case of MVs obtained from Saos-2 cell cultures. These findings suggested that MVs originated from microvilli-like membranes, a specific lipid raft domain due to high enrichment in cholesterol and TNAP and not from endoplasmic reticulum due to lack of CD9 and Alix marker. To determine the types of lipids essential for mineralization, we analyzed Fatty Acids (FAs) in MVs, microvilli and in membrane fractions of chondrocytes isolated from femurs of chicken embryos. The FA composition in the MVs was almost identical to that in microvilli, indicating that the MVs originated from microvilli. These fractions contained more monounsaturated FAs especially oleic acid than in membrane homogenates of chondrocytes. They were enriched in 5, 8, 11-eicosatrienoic acid (20:3*n* – 9), in eicosadienoic acid (20:2*n* – 6), in arachidonic acid (20:4*n*–6). In contrast, membrane homogenates from chondrocytes were enriched in 20:1n-9, 18:3n-3, 22:5n-3 and 22:5n-6. Due to their relatively high content in MVs and to their selective recruitment within microvilli from where MV originate, we concluded that 20:2n-6 and 20:3n-9 (pooled values), 18:1n-9 and 20:4n-6 are essential for the biogenesis of MVs and for bone mineralization.

2nd INTERNATIONAL CONFERENCE ON MATRIX VESICLES : FROM BIOCHEMISTRY TO CLINIC

POSTERS

ICMV 2019

Mineralization-competent osteoblasts require NCX3 for Ca^{2+} entry into matrix vesicles

[1]Irshad A. Sheikh, [1]Monica Midura-Kiela, [1]Fayez K. Ghishan and [1,2]Pawel R. Kiela.
[1]Department of Pediatrics, University of Arizona, Tucson, AZ, United States
(irshad@email.arizona.edu)
[2]Department of Immunobiology, University of Arizona, Tucson, AZ, United States

Matrix vesicles (MVs) provide the initial site of mineralization and an optimum microenvironment for amorphous hydroxyapatite (HA) formation within mineralizing osteoblasts. Though Na^+/Ca^{2+} exchanger isoform-3 (NCX3, SLC8A3) was presumed to function as major Ca^{2+} transporter for Ca^{2+} extrusion out of osteoblast into the calcifying bone matrix, its presence and functional role in MVs remains unknown. We postulated that NCX3 exploits the Na^+ gradient generated by PiT1 (SLC20A1, Sodium-Dependent Phosphate Transporter 1) to pump Ca^{2+} into the MVs that is required for initial HA formation. Western blot analysis indicated the expression of both NCX3 and PiT1 in MVs released by mineralizing pre-osteoblastic MC3T3-E1 cells while immunogold labeling of NCX3 further validated its localization in MVs. Moreover, treatment of MC3T3-E1 cells with TNFα impaired mineralization with concomitant reduce NCX3 expression both in osteoblasts and the released MVs. Compared to control cells, CRISPR-CAS9 mediated NCX3 knockout in MC3T3-E1 (NCX3 KO) cells led to impaired mineralization, with reduced alizarin red staining and decreased Ca^{2+} deposition as determined colorimetrically. More importantly, this impaired mineralization correlated with a decreased capacity of MVs to promote extracellular HA formation, as shown in a cell-free system with MVs from NCX3 KO cells on collagen-coated coverslips. Furthermore, Energy-Dispersive X-ray Spectroscopy (EDS) analysis of MVs released by NCX3 KO cells showed reduced Ca^{2+} accumulation inside the vesicles as compared to MVs secreted by the control cells. Taken together, these results indicate that NCX3 contributes to Ca^{2+} uptake into the MVs and appear critical for osteoblast-mediated mineralization.

ATOMIC FORCE MICROSCOPY AND X-RAY PHOTOELECTRON SPECTROSCOPY TO INVESTIGATE HUMAN OSTEOARTHRITIC CARTILAGE

[1,2]Ilhem-Lilia Jaabar, [2]Antoine Miche,[1]Kristell Wanherdrick, [3]François-Paul Ehkirch, [1]Francis Berenbaum, [1]Xavier Houard, [2]Jessem Landoulsi

[1]Centre de Recherche Saint-Antoine (CRSA), INSERM UMRS-938 Sorbonne Université, F-75012 Paris, France (ilhem.jaabar@sorbonne-universite.fr)
[2]Laboratoire de Réactivité de Surface (LRS), CNRS UMR-7197 Sorbonne Université, F-75005 Paris, France
[3]Clinique Maussins-Nollet, F-75019 Paris, France

Osteoarthritis (OA) is characterized by an irreversible degradation of articular cartilage and an abnormal remodeling of the subchondral bone. The reorganization of the cartilage-bone interface is decisive in the development and progression of this pathology. We hypothesize that the composition and the organization of the extracellular matrix, particularly at this interface, play a pivotal role in OA progression. In this study, we use X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) to probe the composition and the morphology of human OA cartilage. For this purpose, several procedures of sample preparation were evaluated, including tissue embedding (in paraffin *vs* OCT) and decalcification process (with *vs* without ultrasonic treatment). A methodology developed for bio-organic/inorganic interface and adapted for cartilage is used to extract fine chemical information from XPS data (proteins, proteoglycans, polysaccharides, …). The organization of type II collagen fibrils and their dimensions are determined from AFM images, showing structural evolution with the disease progression. Complementary information regarding OA score is provided by histology. This approach, combining biochemical and physicochemical characterizations, provides a relevant way to probe the evolution of the cartilage/bone interface during osteoarthritis.

Biomimetic phospholipid vesicles lubricants: Synthesis and multiphysics characterizations. Application in The Joint Diseases

Darragi N[15]., Massardier V[1]., Zouari A[1]., Maniti O[2]., Piednoir A[3]., Girard-Egrot A[2]., Guichardant M[2]., Landoulsi A[5]., Tinland B[4], Trunfio-Sfarghiu A.M[1]

[1]Univ Lyon, INSA, Mechanics of Contacts and Structures Laboratory LaMCoS, UMR 5259, F-69621 Lyon, France (nesrine.raies@insa-lyon.fr)
[2]Univ Lyon, CNRS, Molecular and Supramolecular Chemistry and Biochemistry Institute ICBMS UMR 5246, F-69622 Lyon, France
[3]Univ Lyon, CNRS, Institut Lumière Matière IML UMR 5306, F-69622 Lyon, France
[4]Univ Aix-Marseille, CNRS, Marseille Interdisciplinary Center of nanosciences CINaM UMR 7325, 13288 Marseille, France
[5]Univ Carthage, Laboratory of Biochemistry and Molecular Biology LBBM, FSB, Tunisia

Synoviocytes within the joints synthesize a crucial fluid for joints life, called the synovial fluid, possessing excellent lubricating properties. Thus, recent studies show that the synovial fluid (SF) involves microvesicles filled with a glycoprotein gel and surrounded by stacked lipid bilayers. On this point, biochemical, structural (TEM, CFM) and mechanical (Langmuir, AFM) analysis of SF lipid vesicles of dog samples (surgical waste during surgery on the cruciate ligament) were performed in order to determine their composition with the aim to produce a biomimetic structure. Biomimetic vesicles (BV) were prepared by respecting 'Gel in' SF Protocol described by Sava et al. The mechanical behaviour of biomimetic phospholipid (PL) monolayers was compared to that of DPPC (''gel" phase at 37°C) and POPC (''fluid'' phase at 37°C). From lipidomic assay results, we propose a synovial fluid biomimetic composition (BC) made up of 20% SpH, 8% POPC, 8% SOPC, 16% DPPC, 16% DSPC, 16% PLPC, 16% SLPC. The Langmuir monolayers results show that this BC of PL increases vesicle rigidity comparing it to that of DPPC. Using this BC, micrometric size vesicles (1 to 10µm) with a multilamellar membrane were obtained similar to those observed directly in the collection of healthy synovial fluid and that obtained using DPPC. BV structure is significantly different from POPC that exhibit an unstable diameter with a strong propensity to aggregate. The nano-mechanical and tribological results reveal that BV structure significantly increases the vesicular stiffness and the lubricant viscosity, which may explain the biolubricant performances of these structures in vivo.

Characterization and properties of vesicles released from chondrocytes

[1]Rene Buchet, [1]Thierry Granjon T, [1]David Magne, [2]Agnieszka Strzelecka-Kiliszek , [3]Joanna Bandorowicz-Pikula, [2]Slawomir Pikula , [4]Pietro Ciancaglini, [4]Mayté Bolean, [5]José Luis Millan, [5,6]Massimo Bottini M and [1]Saida Mebarek

[1]ICBMS UMR 5246 CNRS Universite Lyon 1, Villeurbanne, France France (rbuchet@univ-lyon1.fr). [2]Laboratory of Biochemistry of Lipids and [3]Labolatory of Cellular Metabolism, Nencki Inst. Exp. Biol., Polish Academy of Sciences, Warsaw, Poland. [4]Dept. de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil. [5]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037, USA. [5,6]Department of Experimental Medicine, University of Rome Tor Vergata, Italy.

Chondrocytes release exosomes, vesicles, matrix vesicles (MVs) and apoptotic bodies. Their characterization and properties are the object of intense scrutiny due to their possible roles in initiating mineralization, in cell-to-cell communications. Extracellular-vesicle treatment may be amenable to multiple forms of delivery (injection, impregnated scaffolds) [1]. Among, the difficulties encountered are the purification of one class of micro particles [2]. We compared different methods to extract micro particles and analyzed their properties, using recent findings on properties of micro particles. Due to the strong affinity of MVs to collagen, collagenase treatment allowed to release a large amount of MVs with high tissue-nonspecific alkaline phosphatase (TNAP) and strong ability to initiate apatite formation. Collagenase treatment may impact the integrity of MVs [2], prompting the necessity to develop other methods to extract micro particles. Extracellular media from murine primary chondrocytes, without any treatment of collagenase were subjected to two-step-ultracentrifiguation. The first step of ultacentrifugation allowed to extract the large apoptotic bodies, while the second step gave rise to exosomes and other vesicles. MVs, extracted after collagenase treatment, did not originate from endoplasmic reticulum as indicated by their lack of CD9 and Alix as probed by Western Blot. MVs had similar lipid composition as microvilli-like plasma membrane. Exosomes were evidenced by the presence of CD9 and claudin, while apoptotic bodies did not reveal any CD9 and claudin. Apoptotic bodies or exosomes extracted from chondrocytes were less efficient to mineralize than MVs. These findings confirmed that MVs are distinct from exosomes or apoptotic bodies.

[1] M.C. Blaser and E. Aikawa. Roles and regulation of extracellular vesicles in cardiovascular mineral metabolism. Frontiers in cardiovascular medicine. doi: 10.3389/fcvm.2018.00187[2] R.E. Wuthier, J.E. Chin, J.E. Hale, T.C. Register, L..V. Hale and Y.Ishikawa. Isolation and characterization of calcium-accumulating matrix vesicles from chondrocytes of chicken epiphyseal growth plate cartilage in primary culture. J. Biol. Chem 1985, 260 15972

Fluorescence monitoring of Annexin VI localization in Matrix Vesicles mimetic membranes depending on pH and membrane lipid composition

Wang Y[1], Maniti O[1], Strzelecka-KiliszekA[2], Bandorowicz-Pikula J[3], PikulaS[2], Veschi E[4], Ciancaglini P[4],Bolean M[4], Ramos AP[4], Millan JL[5], Bottini M[5,6], Tian H[7], He X[7], Magne D[1],Buchet R[1], Mebarek S[1], and Granjon T [1]

[1]ICBMS UMR 5246 CNRS University Lyon 1, Villeurbanne, France. [2]Laboratory of Biochemistry of Lipids and [3]Labolatory of Cellular Metabolism, Nencki Inst. Exp. Biol., Polish Academy of Sciences, Warsaw, Poland. [4]Dept. de Química, Faculdade de Filosofia, Ciências e Letras de RibeirãoPreto da Universidade de São Paulo, RibeirãoPreto, São Paulo, Brazil.[5]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037, USA. [5,6]Department of Experimental Medicine, University of Rome Tor Vergata, Italy.[7]Key Laboratory for Advanced Materials and Joint International Research Laboratory of Precision Chemistry and Molecular Engineering, Feringa Nobel Prize Scientist Joint Research Center, East China University of Science and Technology, Shanghai 200237, China. (sakuranokuniwyb@icloud.com)

Matrix vesicles (MVs) are 100-300 diameter spherical bodies released mineral forming cells such as chondrocytes, osteoblasts and ondotoblasts. MVs are often found associated with calcium phosphate mineral crystals and are thought to initiate mineralization. Compared with plasma membrane, from which they are supposed to derive, MVs are enriched in tissue non-specific alkaline phosphatase, phosphatidyl serine (PS) and annexins (Anx). AnxA6 is the largest member of the annexin family, which interacts in a Ca^{2+} dependent manner with negatively charged phospholipid of the MV membrane. It was found to be present in the MVs lumen, on the surface of the membrane internal leaflet, on the membrane external leaflet and also inserted in the bilayer membrane. AnxVI multiple roles, during MVs inducing apatite nucleation and their binding properties to phospholipids as well to collagen are not comprehensively delineated. Using membrane fluorescent probes and proteoliposomes containing either DMPC or DMPC-DPPS (9-1) to mimic external and internal MV membrane leaflet, respectively, we confirmed distinct binding behavior of AnxA6 to MV membranes depending both on the pH drop occurring during apatite formation, and charges in the phospholipid composition, differing between the outer and the inner leaflet of the membrane. These findings suggest that distinct localization of AnxA6 are related to different functions during the mineralization process.

Towards a new drug delivery strategy using membrane physicochemical properties modulations

[1]**J Bompard,** [2]L Brizuela, [2]S Mebarek, [2]T Granjon, [3]AM Trunfio-Sfarghiu, [1]A Girard-Egrot, [1]O Maniti

[1]Univ Lyon, Université Lyon 1, CNRS, ICBMS UMR 5246, GEMBAS team, Lyon, France (julien.bompard@etu.univ-lyon1.fr)

[2]Univ Lyon, Université Lyon 1, CNRS, ICBMS UMR 5246, MEM[2] team, Lyon, France

[3]Univ Lyon, INSA Lyon, CNRS, LaMCoS UMR 5259, TMI team, Lyon, France

Lipid-based carriers such as liposomes are commonly used in drug delivery, and their specificity often relies on a ligand-receptor interaction. However, physicochemical properties of the membrane such as the order degree have never been used in drug delivery, despite reported differences between tumoral and non-tumoral cells.

Using liposomes covering a wide range of membrane fluidity, we assessed their interaction with human prostatic cells coming either from 3 metastatic tumoral cell lines or from a non-tumoral, control cell line. By modulating the liposome composition, we were able to successfully target a particular cell type. Liposomes in a fluid state target tumoral cells while liposomes in a rigid state target control cells. Furthermore, using two different fluorophores, we determined that the mechanism of this interaction is based on fusion of the liposome with the cell membranes.

Hence, using membrane fluidity modifications as a target when designing lipid drug carriers is a promising alternative to current targeting strategies based on covalent grafting.

2nd INTERNATIONAL CONFERENCE ON MATRIX VESICLES : FROM BIOCHEMISTRY TO CLINIC

List of participants

Surname	Name	Affiliation	Email
Abousalham	Abdelkarim	University of Lyon1 ICBMS, Lyon, France	abousalham@univ-lyon1.fr
Amadeu de Oliveira	Sylvia	Sanford Burnham Prebys Medical Discovery Institute, La Jolla, USA	famadeu@sbpdiscovery.org
Arhab	yani	University of Lyon1 ICBMS, Villeurbanne, France	yani.arhab@univ-lyon1.fr
Aydin	Meriem	University of Lyon1 ICBMS, Villeurbanne, Fance	meryem.aydin@etu.univ-lyon1.fr
Berot	Anne	University of Lyon1 ICBMS, Villeurbanne, Fance	anna.berot@insa-lyon.fr
Bessueille	Laurence	University of Lyon1 ICBMS, Villeurbanne, Fance	laurence.bessueille@univ-lyon1.fr
Bolean	Mayte	University of São Paulo, Brazil.	maytebolean@yahoo.com.br
Bompard	Julien	University of Lyon1 ICBMS, Villeurbanne, France.	julien.bompard@etu.univ-lyon1.fr
Borel	Mathieu	University of Lyon1 ICBMS, Villeurbanne, France.	mathieu.borel@univ-lyon1.fr
Bottini	Massimo	Department of Experimental Medicine, University of Rome Tor Vergata	mbottini@sbpdiscovery.org
Bougault	Carole	University of Lyon1 ICBMS, Villeurbanne, France.	carole.bougault@univ-lyon1.fr
Briolay	Anne	University of Lyon1, ICBMS, Villeurbanne, France	anne.briolay@univ-lyon1.fr
Brizuela Madrid	Leyre	University of Lyon1 ICBMS, Villeurbanne, France.	leyre.brizuela-madrid@univ-lyon1.fr
Buchet	René	University of Lyon1 ICBMS, Villeurbanne, France.	rbuchet@univ-lyon1.fr
Ciancaglini	Pietro	University of São Paulo, Brazil.	pietro@ffclrp.usp.br
Como	Juna	University of Lyon1 ICBMS, Villeurbanne, France.	juna.como@univ-lyon1.fr
Darragi Raies	Nesrine	Contact and Structure Mechanics Laboratory "LaMCoS"INSA, Lyon, France.	nesrine.raies@insa-Lyon.fr

Dobrawa	Napierala	Center for Cranio-facial Regeneration, Department of Oral Biology McGowan Institute for Regenerative Medicine School of Dental Medicine University of Pittsburgh	don11@pitt.edu
During	Alexandrine	EA 4490 - Lab. De physiopathologie des maladies osseuses inflammatoires. Université de Lille, Faculté de chirurgie dentaire, Lille, France.	alexandrine.during@univ-lille.fr
El Jamal	Alaeddine	University of Lyon1 ICBMS, Villeurbanne, France.	alaeddine.el-jamal@univ-lyon1.fr
Ferriz	Hortense	University of Lyon1 ICBMS, Villeurbanne, France.	hortenseferriz@hotmail.fr
Ganot	Philippe	Centre Scientifique de Monaco, Monaco	pganot@centrescientifique.mc
Girard	Dorothée	UMRS-MD 1197 " Interactions between stem cells and their niches in physiology, tumors and tissue repair", Clamart, France	dorothee.girard@inserm.fr
Granjon	Thierry	University of Lyon1 ICBMS, Villeurbanne, France.	thierry.granjon@univ-lyon1.fr
Grochocka	Lilianna	Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland.	l.grochocka@nencki.gov.pl
Guillot	Thomas	University of Lyon1 ICBMS, Villeurbanne, France.	Thomas.guillot452@gmail.com
Hannoun	Amira	LaMCoS - INSA Villeurbanne, France.	amira.hannoun@insa-lyon.fr
Jaabar	Ilhem	University of Lyon1 ICBMS, Villeurbanne, France	ilhemlilia.jaabar@gmail.com
Kawtharany	Line	University of Lyon1 ICBMS, Villeurbanne, France.	lynn.kawtharany@etu.univ-lyon1.fr

Magne	David	University of Lyon1 ICBMS, Villeurbanne, France.	david.magne@univ-lyon1.fr
Maniti	Ofélia	University of Lyon1 ICBMS, Villeurbanne, France.	ofelia.maniti@univ-lyon1.fr
Mebarek	Saida	University of Lyon1 ICBMS, Villeurbanne, France.	saida.mebarek@univ-lyon1.fr
Millàn	José luis	SANFORD BURNHAM PREBYS Medical Discovery Institute La Jolla, California	millan@sbpdiscovery.org
Ramos	Ana Paula	University of São Paulo, Brazil.	anapr@ffclrp.usp.br
Randrianalisoa-touvent	Florina	University of Lyon1 ICBMS, Villeurbanne, France	florina.randrianalisoa-touvent@etu.univ-lyon1.fr
Sfarghiu	Ana-maria	Contact and Structure Mechanics Laboratory "LaMCoS"INSA, Lyon, France.	Ana-maria.Sfarghiu@insa-lyon.fr
Sheikh	Irshad Ali	Department of Pediatrics, University of Arizona, United States	irshad@email.arizona.edu
Strzelecka-Kiliszek	Agnieska	Nencki Institute of Experimental Biology, Polish Academy of Sciences, Varsaw, Poland	a.strzelecka kiliszek@nencki.gov.pl
Tambutté	Sylvie	Centre Scientifique de Monaco, Monaco	stambutte@centrescientifique.mc
Wang	Yubie	University of Lyon1 ICBMS, Villeurbanne, France	sakuranokuniwyb@icloud.com