Congrès national annuel de la Société Française de Biologie de la Matrice Extracellulaire

Toulouse, France

Amphi. Gallais - LCC
205 route de Narbonne

22-23 sept. 2025

SFBMEc

Toutes les informations :
sfbmec2025.sciencesconf.org

Sommaire

A propos de SFBMEc 2025

La **SFBMEc** est une société savante française dédiée à la promotion de la recherche et de la diffusion des connaissances dans le domaine de la **biologie de la matrice extracellulaire**.
Ses membres (scientifiques, médecins, pharmaciens, dentistes, vétérinaires) proviennent des différents laboratoires et disciplines (biochimie, biologie cellulaire, biologie moléculaire et biophysique).
Ces disciplines sont utilisées pour l'étude de la matrice extracellulaire dans de nombreux domaines tels que **la cancérologie, la cardiologie, la dermatologie, le développement, l'hépatologie et la rhumatologie**.

La SFBMEc organise depuis plus de 30 ans des journées scientifiques qui rassemblent les principaux acteurs de la matrice extracellulaire en France, et plus marginalement en Europe.

En 2025, le congrès national annuel se tient les 22 et 23 septembre, à Toulouse.

Ces rencontres se déroulent habituellement sur 2 jours, et la première demi-journée est organisée par et pour les jeunes chercheurs (doctorants et post-doctorants) réunis au sein de la SFBMEc Avenir.

Les thématiques principales

Le programme scientifique de ces journées en 2025 est structuré autour de 3 thématiques majeures :

>> ECM & cancer/physiopathologie, mettant en lumière son implication dans divers cancers, maladies chroniques et inflammatoires.

>> Assemblage & dynamique de l'ECM, focalisée sur les processus fondamentaux de construction et remodelage de la matrice extracellulaire.

>> ECM & technologies pour la santé, explorant les mécanismes moléculaires et les applications thérapeutiques.

Les conférenciers et conférencières invités

>> Session 1 "ECM & cancer / physiopathologie"

Emmanuel Donnadieu, Institut Gustave Roussy Paris

"Extracellular matrix as a barrier to CAR T cell therapy in solid tumors"

Soline Estrach, IRCAN Nice

"Epidermal stem cell activation: A history of extracellular matrix assembly"

>> Session 2 "Assemblage & dynamique de l'ECM"

Florence Ruggiero, ENS Lyon

"From a collagenocentric to a more integrated view of matrix biology in development, repair and disease"

Jean-Daniel Malcor, LBTI Lyon

"Peptidic models of the collagen triple helix"

>> Session 3 "ECM & technologies pour la santé"

Nicolas L'Heureux, BioTis Bordeaux
"Cell-assembled extracellular matrix (CAM) for the production of human textiles"

Gwendal Josse, Pierre Fabre Toulouse
"Biophysical investigation of skin structure"

Stéphanie Baud, MEDyC Reims
"Molecular modelling, virtual reality and AI: real assets for observing, understanding and deciphering the multi-scale interactions of the ECM"

Le comité scientifique

Le comité scientifique est composé de :

> Laure **Gibot** / Softmat, Toulouse
> Valérie **Samouillan** / CIRIMAT, Toulouse
> Jany **Lods** / CIRIMAT, Toulouse
> Sophie **Girod Fullana** / CIRIMAT, Toulouse
> Catherine **Moali** / LBTI, Lyon
> Emeline **Groult** / IRMB, Montpellier

Le comité d'organisation

Le comité d'organisation est composé de :

> Laure **Gibot** / Softmat, Toulouse
> Fanny **Weiss** / Softmat, Toulouse
> Valérie **Samouillan** / CIRIMAT, Toulouse
> Jany **Lods** / CIRIMAT, Toulouse

Partenaires de SFBMEc 2025

L'organisation d'un tel évènement scientifique ne peut être assurée qu'avec le concours de partenaires publics et privés, que nous remercions vivement !

En particulier, il a été organisé en étroite collaboration avec le laboratoire Softmat

et avec l'aimable accueil du laboratoire LCC.

>> Partenaires Or

>> Partenaires Argent

>> Partenaires Bronze

GDR Biomim - 2088

Programme SFBMEc 2025

Horaires	Lundi 22/09
SESSION SFBMEc Avenir	
08h50	**Introduction**
09h00	**Communications orales** 9h00 : Lorinne Adam 9h20 : Louise Benarroch 9h40 : Alexis David
10h00	**Short talks**
10h40	***Pause café***
11h00	Projection dans le métier de chercheur par **Guillaume Sire** - *Université Toulouse Capitole*
12h00	***Déjeuner cocktail***
13h00	**Accueil des participants**
13h15	**Ouverture du congrès**
SESSION 1 : ECM & cancer/physiopathologie	
13h30	**Emmanuel Donnadieu** - *Institut G. Roussy Paris*
14h00	**Communications orales** 14h00 : Gaëtan Thivolle Lioux 14h15 : Fabien Foucher 14h30 : Justine Bourny 14h45 : Céline Schmitter
15h00	***Pause café & Session posters***
16h00	**Soline Estrach** - *IRCAN Nice*
16h30	**Communications orales** 16h30 : Lucile Cadoret 16h45 : Carine Le Goff 17h00 : Amina Boukhobza
17h15	**Temps libre**
17h30	**Départ pour le centre de Toulouse**
18h25	***Balade apéritive sur la Garonne***
20h00	***Dîner de gala***

Fin de la journée

Horaires	Mardi 23/09
SESSION 2 : Assemblage & dynamique de l'ECM	
08h30	**Keynote de Florence Ruggiero** - *ENS Lyon*
09h15	**Communications orales** 09h15 : Dimitrios Kourtzas 09h30 : Alexandra Pavilla 09h45 : Violaine Sée 10h00 : Jean-Baptiste Vincourt
10h15	***Pause café & Session posters***
11h00	**Jean-Daniel Malcor** - *LBTI Lyon*
SESSION 3 : ECM & technologies pour la santé	
11h30	**Communications orales** 11h30 : Anaïs Lavrand 11h45 : Jeanne Minvielle Moncla
12h00	**Assemblée Générale de la SFBMEc**
13h00	***Déjeuner cocktail & Session posters***
14h00	**Nicolas L'Heureux** - *Biotis Bordeaux*
14h30	**Communications orales** 14h30 : Chayma Saadan 14h45 : Gaëlle Rousselet & Alexandre Raoul
15h00	**Gwendal Josse** - *Pierre Fabre Toulouse*
15h30	**Stéphanie Baud** - *MEDyC Reims*
16h00	**Cérémonie de remise des prix & clôture du congrès**

Fin de la journée

Programme complet

SESSION SFBMEc Avenir

An eco-friendly implantable medical device derived from perinatal tissues treated with supercritical carbon dioxide

Lorinne Adam [a,b], Halima Kerdjoudj [b], Solène Rota [a], Raphaël Bardonnet [a], Nicole Bouland [b].

[a] *Society BIOBank, Lieusaint, France*
[b] *UR 4691 BIOS, Reims, France*

Keywords: perinatal tissues; supercritical carbon dioxide; sterilization; preservation

Abstract:

INTRODUCTION
Successful commercialization of any tissue product depends on the effective preservation of key biological components essential to maintaining its intended therapeutic function. Perinatal tissues (PTs), such as the amniotic membrane and umbilical cord, hold significant promise for wound healing due to their richness in growth factors and other bioactive molecules[1,2]. Used as early as World War I to treat deep injuries, PTs—considered immune-privileged tissues with a reduced risk of rejection—are generating increasing interest, particularly in ophthalmology and regenerative medicine. One of the main limitations of PTs as a tissue source is their transient availability, as they exist in an active form only during a short period immediately postpartum. Supercritical carbon dioxide ($scCO_2$), obtained at 31°C, is already used to preserve bone grafts (e.g., BIOBank)[3]. This CIFRE PhD project aims to evaluate the impact of $scCO_2$ treatment on PT matrix integrity, with the objective of enabling their long-term preservation.

METHODOLOGY
Perinatal tissues were collected from the Reims Maternity Hospital under authorization from the "Cellule Bioéthique" (DC-2014-2262) and processed under sterile conditions in a laminar flow hood. The amniotic membrane (AM), Wharton's jelly (WJ), and placental-derived umbilical cord membranes (AMc) were carefully isolated, packaged in double Tyvek® bags, and treated with $scCO_2$ using the Supercrit® process (BIOBank). Freeze-dried PTs served as controls. Structural, physicochemical, and biological analyses were performed on a minimum of four samples and analyzed statistically using GraphPad Prism.

RESULTS AND DISCUSSION
Histological analysis (H&E staining and DAPI labeling) confirmed effective devitalization of $scCO_2$-treated PTs, comparable to freeze-dried controls. Scanning electron microscopy revealed a marked reduction in tissue thickness following $scCO_2$ treatment—approximately 10-fold, 6-fold, and 3-fold for WJ, AMc, and AM, respectively, compared to controls ($p < 0.0001$). Despite their initially high hydration levels, $scCO_2$-treated PTs exhibited a significant decrease in swelling capacity (~3-fold) and porosity (~1.3-fold) compared to controls ($p < 0.0001$). Biochemical assays indicated a substantial loss of collagen and glycosaminoglycans, especially hyaluronic acid (~5-fold reduction, $p < 0.0001$), a key molecule for hydration. Importantly, $scCO_2$-treated PTs were non-cytotoxic (in compliance with ISO 10993-5), retained hemostatic and anti-inflammatory properties, and supported dermal fibroblast adhesion and proliferation, despite a decrease in growth factor content.

CONCLUSION
Supercritical CO_2 treatment of perinatal tissues represents an innovative, efficient, and environmentally friendly method for their long-term preservation. Validation studies are ongoing, with particular focus on applications in ophthalmology (pending ANSM approval).

Reference:

[1] Deus et al. « Perinatal Tissues and Cells in Tissue Engineering and Regenerative Medicine », [2] Gindraux et al., « Perinatal Derivatives Application », [3] Mitton, Rappeneau, et Bardonnet, « Effect of a Supercritical CO_2 Based Treatment on Mechanical Properties of Human Cancellous Bone ».

LAMA2-related muscular dystrophy: a novel cellular model to investigate pathophysiological mechanisms

Louise Benarroch[a*], Lauren Struble[a], Kamel Mamchaoui[a], Anne Bigot[a], Nur Villar-Quiles[a,b], Tanya Stojkovic[a,b], Valérie Allamand[a]

[a]*Sorbonne Université, Inserm, Institut de Myologie, Centre de Recherche en Myologie, Paris, France*
[b]*APHP, Centre de référence de pathologie neuromusculaire, FILNEMUS, Institut de Myologie, Département de Neurologie, GH Pitié Salpêtrière, Paris, France*

* louise.benarroch@inserm.fr

Keywords: Neuromuscular disorders, Laminins, Cellular models, Myomatrix

Abstract:

Congenital muscular dystrophies (CMD) correspond to a large group of heterogeneous neuromuscular disorders characterized by an early onset (at birth or first months of life). One of the most common form of CMDs in Europe is the Laminin α2-related CMDs (LAMA2-CMD) due to mutations in the LAMA2 gene [1]. This gene codes for the alpha-2 chain of Laminin 211, a trimeric protein localized at the basal membrane surrounding muscle fibers [2]. Complete LAMA2 deficiency is associated with a more severe phenotype, known as merosin-deficient congenital muscular dystrophy type 1A (MDC1A), than partial LAMA2 deficiency, causing a milder, childhood- or adult-onset limb-girdle type muscular dystrophy [3]. LAMA2-patients display muscle weakness, hypotonia, proximal joint contractures and respiratory dysfunction, the main cause of death with 30% of patients dying in the first decade of life.

To explore the mechanisms underlying LAMA2-CMD or more globally neuromuscular disorders, in particular myogenesis and muscle-related pathways on myoblasts from patients or from healthy subjects can pose ethical and procedural challenges that limit such investigations. To obtain myogenic cells, a muscle biopsy needs to be performed. However, due to the invasiveness of the procedure, it became a rare occurrence in a diagnostic setting. And practically, skin biopsies (less invasive) are preferred over muscle biopsies, making skin fibroblasts the only cell type available. Therefore, converting skin fibroblasts into myogenic cells by forcing the expression of the myogenic regulator MYOD can be an alternative system to myoblast differentiation and allow us to investigate muscular dystrophies [4].

Using myo-converted fibroblasts form 3 patients, both carrying homozygous LAMA2 mutations, we are investigating and characterizing several mechanisms, related to myogenesis and related-pathways that may be impaired in myogenic cells of LAMA2-patients.

References:

[1] H Kölbel, D Hathazi, M Jennings, et al., Frontiers in Neurology, 2019, **10: 470**.
[2] L Shaw, CJ Sugden and KJ Hamill, *Frontiers in Genetics*. 2021, **12:707087.**
[3] A Sarkozy, AR Foley, AA Zambon et al., *Frontiers in Molecular Neuroscience,* 2020, **13:123**
[4] L Benarroch, J Madsen-Østerbye, M Abdelhalim, et al., *Cells*. 2023, **12(15):1995**

Inhibition of cathepsin S by synthetic chondroitin 4-sulfate oligosaccharides

Alexis David[a,b], Florian Surback[a,b], Roxane Domain[a,b], Clément Boutet[a,b], Aude Vibert[c], Pierre Buisson[c], Martyna Maszota-Zieleniak[d], Ludovic Landemarre[e], Marie Schuler[c], Gilles Lalmanach[a,b], Sergey A Samsonov[d], Chrystel Lopin-Bon[c], Fabien Lecaille[a,b*]

[a] University of Tours, Tours, France.
[b] INSERM, UMR1100, Research Center for Respiratory Diseases (CEPR), Team Proteolytic enzymes and their pharmacological targeting in lung diseases, Tours, France.
[c] University of Orléans, CNRS, UMR 7311, Institut de Chimie Organique et Analytique (ICOA), Orleans, France.
[d] Faculty of Chemistry, University of Gdansk, Poland.
[e] GLYcoDIAG, Orléans, France

* fabien.lecaille@univ-tours.fr

Keywords: Cysteine protease, extracellular matrix, glycosaminoglycans, inhibitor, proteolysis

Abstract:
Cathepsin S (Cat S) is a cysteine protease involved in several human diseases (i.e., autoimmune, inflammatory and cardiovascular disorders, cancer, and psoriosis) and is an important target in drug development [1]. We previously reported that Cat S was selectively inhibited by mammalian chondroitin 4 sulfate (C4S) [2, 3]. Chondroitin sulfates (CS) are long linear negatively glycosaminoglycans (GAGs), composed of repeating β-D-glucuronic acid (GlcA) and *N*-acetyl-β-D-galactosamine (GalNAc) units arranged in the sequence by GlcA-β(1→3)-GalNAc-β(1→4) glycosidic bonds, with variable high and low sulfation patterns [4]. Nevertheless, current sources of CS from extracts of tissues of animal origin, do not guarantee sufficient homogeneity and purity to fully reproduce their biological activities nor for therapeutic application. An alternative is the development of synthetic oligosaccharides derived from CS, having the same characteristics as their counterpart of animal origin. Here, synthetic biotinylated non- or sulfated chondroitin oligomers were synthesized and evaluated for their property to inhibit Cat S. The disaccharide unit of C4S (C4S dp2) displayed *in vitro* potent inhibitory activity toward Cat S with IC_{50} value in the micromolar range and showed selectivity over cathepsins K and L. Molecular dynamic studies suggested that only C4S dp2 occupies selectively the region of the active site of Cat S. In addition, we found that multivalent C4S dp2 was much more efficient in Cat S inhibition (nanomolar range) than its monovalent counterpart. This study reports a new approach for designing selective and potent inhibitors of Cat S using multivalent chondroitin sulfate derivatives as a molecular platform.

References:
[1]: F. Lecaille ; J. Kaleta ; D. Brömme. *Chem. Rev.,* 2002, **102**, 4459-4488.
[2]: J. Sage ; F. Mallèvre ; F. Barbarin-Costes ; SA. Samsonov ; JP. Gehrcke ; MT. Pisabarro ; E. Perrier E ; S. Schnebert ; A. Roget ; T. Livache, C. Nizard ; G. Lalmanach ; F. Lecaille. *Biochemistry*, 2013, **52(37)**:6487-98.
[3]: K. K. Bojarski ; A. David ; F. Lecaille ; S.A. Samsonov. *Carbohydr Res.,* 2024, 543, 109201.
[4]: A. David ; T. Chazeirat ; A. Saidi ; G. Lalmanach ; F. Lecaille. *Biomedicines*, 2023, **11**, 810.

SESSION 1

"ECM & cancer / physiopathologie"

Conférences invitées

Extracellular matrix as a barrier to CAR T cell therapy in solid tumors

Emmanuel Donnadieu[a, b]

[a] *INSERM UMR-1186, Gustave Roussy*
[b] *Equipe Labellisée Ligue contre le cancer*

* emmanuel.donnadieu@inserm.fr

Keywords: cancer, immunotherapy, ex vivo human model, ECM

Abstract:
Cancer immunotherapies, particularly those based on adoptive T cell transfer like CAR T cells, have revolutionized the treatment of certain cancers, especially hematologic malignancies. However, these strategies do not yet work effectively in solid tumors, and a major challenge is to determine the mechanisms behind these failures in solid cancers.

To address this, our laboratory has developed an ex vivo experimental system using tumor slices kept viable, which combined with fluorescence imaging allows us to directly track CAR T cell function and identify several obstacles to their antitumor activation, particularly their migration and interactions with tumor cells.

A few years ago, we showed that in lung tumors, extracellular matrix proteins, due to their distribution and orientation, can actually prevent T cells from migrating and forming contacts with tumor cells [1]. We also highlighted certain approaches that target these matrix proteins, particularly those targeting the cross-linking of collagen fibers [2]. These inhibitors allowed us to confirm the importance of the extracellular matrix in immunotherapies, including anti-PD-1 therapies [2].

Our current work aims to understand how the extracellular matrix influences CAR T cell function. At this congress, I will present projects involving anti-B7H3 CAR T cells in human liposarcoma explants, which also suggest that the structure of the extracellular matrix influences the mode of action of CAR T cells.

Extracellular matrix (orange) restricts CAR T cell (green) access to tumor cells (blue, left). Matrix disruption facilitates CAR T cell infiltration and tumor targeting (right).

References:
[1]: Salmon H et al.. (2012). J. Clin. Invest. 122(3):899-910. doi: 10.1172/JCI45817
[2]: Nicolas-Boluda A et al. (2021) Elife 10. doi: 10.7554/eLife.58688

Epidermal stem cell activation: A history of extracellular matrix assembly

Soline Estrach[1], Charles-Maxime Vivier[1], Laurence Cailleteau[1], Ludovic Cervera[1], Kim B Jensen[2,3] and Chloé C. Féral[1]

[1] *Université Côte d'Azur, INSERM, CNRS, IRCAN, 06107 Nice, France.*
[2] *BRIC - Biotech Research and Innovation Centre, University of Copenhagen, Ole Maaloes Vej 5, DK-2200 Copenhagen N, Denmark*
[3] *Novo Nordisk Foundation Center for Stem Cell Research, Faculty of Health and Medical Sciences, University of Copenhagen, DK-2200 Copenhagen N, Denmark*

soline.estrach@inserm.fr

Keywords: Fibronectin, Hair Follicle stem cells, Mechanotransduction signalling pathway

Abstract:

To investigate physiological tissue regeneration, we use the mouse hair follicle[1], which cycles between phases of growth and regression while maintaining a pool of stem cells (HFSC) to sustain tissue regeneration. HFSC via niche interactions fuel the hair regeneration cycle. Regulatory signals that balance SC quiescence and activity are provided by the niche. As a permanent part of the niche, extracellular matrix (ECM) components are key players of its instructive power[2–5]. Even though, cell fate decisions are affected by signals from the ECM within the stem cell niche, how ECM composition and signalling are regulated remains unclear.

Fibronectin (FN) is a major extracellular matrix component and integrin ligand, which role is well characterized during wound healing in skin. Here, using the hair follicle as a mini organ which regenerates, we discovered a novel and unprecedented role for FN in epidermal regeneration. Hair follicle stem cells (HFSCs) undergo long-term self-renewal and multi-lineage differentiation. We show that FN displays a highly specific enrichment in stem cells at the onset of hair follicle regeneration. We reveal FN tracks along the regenerating hair follicles forming a meshwork. FN conditional deletion in HFSC compartments (Lrig1, K19) leads to impaired stem cell location and fate. Loss of this meshwork is accompanied by hair regeneration blocade. Dermal injection of exogenous FN rescues these phenotypes.

SLC3A2, an essential enhancer of integrin signaling and matrix assembly, regulates skin homeostasis. Epidermal SLC3A2 is required for SC population maintenance, establishment and location. SLC3A2 expression in the HFSC compartment is essential for stem cell fate decisions.

Finally, we found that FN/SLC3A2/integrin mechanotransduction cascade finely tunes adult stem cell fate and tissue regenerative power.

References:

1. L. Alonso & E.Fuchs. The hair cycle. *J. Cell. Sci.* **119**, 391–393 (2006).
2. J. C. Adams & F. M Watt. Fibronectin inhibits the terminal differentiation of human keratinocytes. *Nature* **340**, 307–309 (1989).
3. N. Liu *et al.* Stem cell competition orchestrates skin homeostasis and ageing. *Nature* **568**, 344–350 (2019).
4. L. Lukjanenko. *et al.* Loss of fibronectin from the aged stem cell niche affects the regenerative capacity of skeletal muscle in mice. *Nat. Med.* **22**, 897–905 (2016).
5. Matsumura, H. *et al.* Hair follicle aging is driven by transepidermal elimination of stem cells via COL17A1 proteolysis. *Science* **351**, aad4395–aad4395 (2016).

Communications orales

Unravelling a new ECM stiffening function of the axon guidance molecule Netrin-1.

THIVOLLE LIOUX Gaëtan[1,2], FATTET Laurent[1], MEHLEN Patrick[1, 3, 4]

[1] Apoptosis, Cancer and Development Laboratory, Cancer Research Center of Lyon (CRCL), Lyon, France

[2] Université Claude Bernard Lyon 1 (UCBL1), Lyon, France

[3] Leon Berard Comprehensive Cancer Center, Lyon, France

[4] Netris Pharma, Lyon, France

gaetan.thivolle@lyon.unicancer.fr *laurent.fattet@lyon.unicancer.fr*

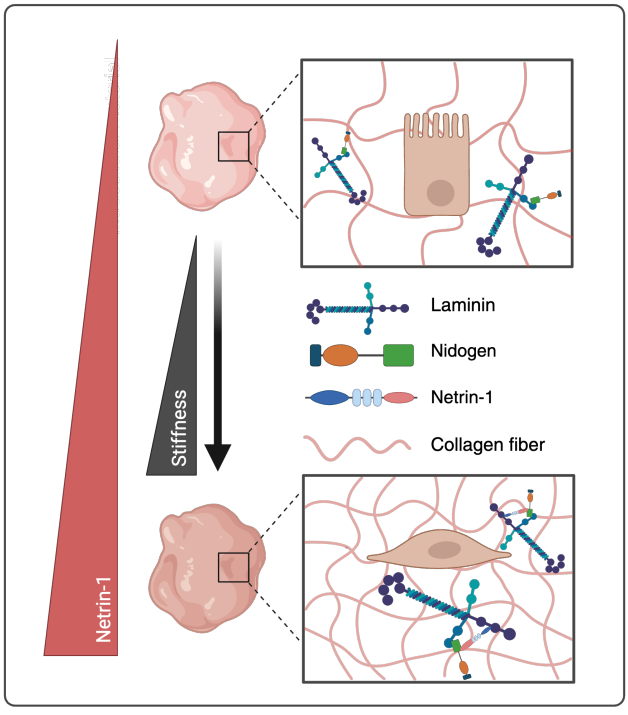
Keywords: ECM remodelling, ECM stiffness, Cancer

Abstract:

The extracellular matrix (ECM) is a complex and dynamic network composed of core structural proteins and matrisome-associated components. Under physiological conditions, ECM homeostasis contributes to the maintenance of natural tissue stiffness. However, disruption of this balance can lead to abnormal stiffening, a hallmark observed in various pathological conditions, including fibrosis and cancer.

Our laboratory investigates the role of the dependence receptor ligand Netrin-1 (NTN1), a neuronal guidance protein predominantly expressed during early developmental stages. Intriguingly, NTN1 is frequently re-expressed in tumors, where its presence is correlated to an increased ECM stiffness. As such, our research aims to elucidate the role of NTN1 in ECM remodelling and stiffening.

Using atomic force microscopy (AFM) on inert Matrigel matrices, we observed a marked increase in stiffness upon supplementation with recombinant Netrin-1 (rNTN1). This stiffening effect was reversed by NP137, a monoclonal antibody targeting NTN1. Structural analyses via immunofluorescence staining for laminin-111 and rNTN1, alongside scanning electron microscopy (SEM), revealed a significant reduction in pore size in the presence of rNTN1. Complementary *in silico* analysis using PEPPI software indicated strong interactions between NTN1 and key ECM components such as Nidogen and Laminin.



Graphical Abstract

To validate these predicted interactions, we performed biolayer interferometry with various ECM proteins. Furthermore, 3D culture models of MCF10A normal mammary epithelial cells exposed to rNTN1 displayed a phenotypic transition toward a more mesenchymal and invasive state. Interestingly, knockdown of NTN1 receptors did not prevent this phenotypic shift, suggesting a novel receptor-independent function for NTN1 in promoting cell invasiveness through direct modulation of ECM stiffness.

Ongoing structural analyses are being conducted on fibroblast-derived matrices, with and without endogenous NTN1 expression, to evaluate its potential remodelling effects on networks of various ECM structural components. In parallel, these matrices are used in functional assays to investigate how the impact of NTN1 on the ECM influences cell behaviours and transcriptional activity, using a range of experimental approaches.

Multimodal Analysis of Fibrous Nests in Human Hepatocellular Carcinomas Using Spatial Transcriptomics, a 62-Antibody Immune Oncology Multiplex Panel and Second Harmonic Generation

Fabien Foucher [a], Tianyuan Wang [a], Maëlle Guillout [b], Gevorg Ghukasyan [b], Marine Seffals [b], Luis Cano [a], Denis Rouède [d], François Tiaho [e], Laurence Dubreil [c], Liang Zhang [f], Romain Désert,[a] Anthony Sébillot [b], Orlando Musso [a]

[a] *INSERM, INRAE, Univ Rennes, Nutrition Metabolisms and Cancer, Rennes, France.*
[b] *Univ Rennes, CNRS, INSERM, UAR Biosit, Core Facility H2P2, France*
[c] *INRAe, Univ Nantes, Oniris, APEX, Cellule d'Expertise en Anatomie Pathologique, France.*
[d] *CNRS, Univ Rennes, Institut de Physique de Rennes, France.*
[e] *INSERM, Univ Rennes, IRSET, Rennes, France.*
[f] *Nanostring Technologies, Bruker, Seattle, USA*

* orlando.musso@univ-rennes.fr

Keywords: tumor microenvironment, desmoplasia, cancer immunity, cancer stem cells

Abstract:

Introduction: Hepatocellular carcinoma (HCC) is the 3rd leading cause of cancer-related death worldwide. Its therapeutic resistance complicates patient management.[1] Microscopic foci of tumor fibrosis, termed *"Fibrous Nests"* are seen in approximately 30% of HCCs.[2-4] Our previous studies showed that tumors containing *fibrous nests* are enriched in fibrillar and matricellular extracellular matrix (ECM) glycoproteins, cancer stem/progenitor cells, WNT/TGF-β activation and immune exhaustion.[3, 5 6] The aim of this study is to refine the cartography of spatial relationships among ECM assemblies, cancer stem/progenitor cells and immune cell populations in HCC tumor tissues.
Methods: We constructed two tissue microarrays (TMAs), comprising a total of 50 HCCs samples, each represented by triplicate 1-mm in diameter cores, derived from archival formalin-fixed paraffin-embedded (FFPE) tissue blocks. Five µm TMA sections were processed for spatial transcriptomics (1000-RNA-plex, Bruker) and multiplex immunohistochemistry (62-antibody-plex immune-oncology panel, Bruker). Quantitative data were obtained from 510x510 µm regions of interest (ROIs) in 1-2 cores per tumor. Machine learning-based cell segmentation was based on nuclear (DAPI) and cell surface (PanCK, B2M, CD45) markers. Cell proximity and trajectory analyses defined cell niches based on their relative enrichment in specific cell populations. Tumor fibrosis was quantitated by QuPath analysis of Sirius Red-stained contiguous TMA sections. A threshold to sort out high-grade from low-grade fibrosis was determined using the elbow method. Collagen fiber packing and orientation were studied by polarized second harmonic generation (P-SHG) on selected samples from this tumor collection.
Results: Quantitative Sirius Red analyses of 91 TMA spots showed that the cutoff discriminating high/low grade fibrosis was 10% of the tissue area: HIGH, median 20%, Q25,17%, Q75, 28%; LOW, median 2%, Q25, 1%, Q75 5%, $P = 5.5^{-14}$. These results were consistent with our recent study on 20 HCCs,[2] which was further confirmed by SHG. P-SHG-based estimation of the mechanical stress index ranged from 2-5 kPa in LOW to 7-24 kPa in HIGH grade fibrosis. Combined spatial transcriptomics and immunoproteomics identified 7 specific cancer cell niches, ranging from cancer stem/progenitor cells to well-differentiated tumor hepatocytes. Proximity analyses showed that the cancer stem/progenitor cell niche was the closest to fibrovascular stalks. Fibrous nests were enriched in myofibrobalsts, tumor-associated macrophages and immune suppressive cells like T regulatory cells and plasmablasts. Trajectory analyses revealed a continuum from the fibrovascular, ECM-rich cancer stem/progenitor cell niche toward differentiating tumor cells.
Conclusion: These data, taken together with our previous matrisome analysis,[3] suggest that ECM assemblies in fibrous nests are key cancer stem cell niches fostering tumor expansion in an immune tolerant microenvironment.

References:

[1] L Cano, F. Foucher et al: Front Oncol Cancer Epidemiology and Prevention 2025, **15**: *in press*.
[2] R Desert, F Foucher F, et al: Hepatology 2024, **79**:E141-E3.
[3] R Desert et al: Hepatology 2023, **78**:741-57.
[4] H Kanzaki et al: Hepatology 2023, **78**:691-3.
[5] R Desert et al: Int J Biochem Cell Biol 2016, **81(Pt A)**:195-207.
[6] R Desert et al: Hepatology 2017, **66**:1502-18.

Characterization of the direct effects of sublethal photodynamic therapy-induced oxidative stress on cutaneous extracellular matrix

Justine Bourny[a,b]*, Bao Doan Giang[a], Zeina Kassem[a], Charles-Louis Serpentini[a], Marie-Hélène Lacoste-Ferré[b,c], Anne-Françoise Mingotaud[a], Patricia Vicendo[a], Valérie Samouillan[b], Laure Gibot[a]

[a] *Université de Toulouse, CNRS UMR 5623, Laboratoire Softmat, Toulouse, France*

[b] *CIRIMAT UMR 5085, Université de Toulouse, Toulouse, France*

c Gérontopôle- CHU Toulouse, 224 Avenue de Casselardit, TSA 40031, 31059, Toulouse Cedex 9, France

justine.bourny@univ-tlse3.fr

Keywords: Oxidative stress, Photodynamic therapy (PDT), Extracellular matrix (ECM), Tissue remodelling

Abstract: Extracellular matrix of the dermis is mainly composed of collagens type I and III, along with elastin, proteoglycans, and glycoproteins. It plays a crucial role in skin structure and physiology. The ECM is a dynamic structure maintained by a balance between its synthesis, maturation, degradation, and recycling of its components. Among the factors capable of influencing this remodelling, reactive oxygen species (ROS) have been described as agents that can directly alter ECM components or modulate the activity of enzymes involved in its remodelling, such as metalloproteinases (1–3).

In our study, we propose to use sublethal photodynamic therapy (PDT) as a tool to locally generate ROS (4). Unlike classical antitumor approach aiming at inducing cell death because of ROS overload, the goal here is **to use photosensitizers to locally and controllably generate ROS, with the intent of non-cytotoxic modulation of the extracellular matrix**. However, the majority of photosensitizers display low aqueous solubility and needs to be encapsulated to improve their photochemical efficiency and increase their cell penetration (5,6). We chose self-assembled block copolymer micelles of the Poly(ethylene oxide -b- ε -caprolactone) (PEO_{5000} PCL_{4000}) and Poly(styrene)-b-poly(ethylene oxide) (PEO_{3100} PS_{2300}) types. The originality of our approach is to understand not only the indirect effects (*via* cellular responses induced by sublethal PDT) on the extracellular matrix, but also the **direct effects of ROS (photochemistry) on matrix components**. It is on this “direct effect” aspect that we are currently concentrating.

First, we performed an exhaustive characterization of the chemical and photochemical properties of the photosensitizers (pheophorbide *a* and hypericin) in their free form and encapsulated one in PEO-PCL and PEO-PS micelles through UV-vis spectroscopy, fluorometry, dynamic light scattering (DLS) and EPR Electron Paramagnetic Resonance (EPR) to detect and quantify ROS species.

Then, we used a combination of physico-chemical approaches to study ECM direct modification by ROS with characterization techniques such as: circular dichroism (CD), Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). The experimental strategy of analysis was first led on easy to analyze simple collagen type I gel model and will then be reconducted using devitalized 3D human tissue-engineered dermal substitutes to more efficiently recapitulates ECM complexity while eliminating the cellular component.

The initial CD results obtained on collagen gels did not reveal any significant effect of ROS and will be complemented by DSC and FTIR studies. Further studies using the more complex and biologically relevant devitalized dermal substitute may provide clearer and more informative results.

References:

1. Svineng G, Ravuri ,Chandra, Rikardsen ,Oddveig, Huseby ,Nils-Erik, and Winberg JO. The Role of Reactive Oxygen Species in Integrin and Matrix Metalloproteinase Expression and Function. Connect Tissue Res. 1 janv 2008;49(3-4):197-202.
2. Hovhannisyan V, Guo HW, Hovhannisyan A, Ghukasyan V, Buryakina T, Chen YF, et al. Photo-induced processes in collagen-hypericin system revealed by fluorescence spectroscopy and multiphoton microscopy. Biomed Opt Express. 2 avr 2014;5(5):1355-62.
3. Gouarderes S, Ober C, Doumard L, Dandurand J, Vicendo P, Fourquaux I, et al. Pulsed Electric Fields Induce Extracellular Matrix Remodeling through Matrix Metalloproteinases Activation and Decreased Collagen Production. J Invest Dermatol. 1 mai 2022;142(5):1326-1337.e9.
4. Zhang Y, Liu W, Wang Q. Positive effects of low-dose photodynamic therapy with aminolevulinic acid or its methyl ester in skin rejuvenation and wound healing: An update. J Biophotonics. 2023;16(4):e202200293.
5. Gibot L, Lemelle A, Till U, Moukarzel B, Mingotaud AF, Pimienta V, et al. Polymeric Micelles Encapsulating Photosensitizer: Structure/Photodynamic Therapy Efficiency Relation. Biomacromolecules. 14 avr 2014;15(4):1443-55.
6. Gibot L, Demazeau M, Pimienta V, Mingotaud AF, Vicendo P, Collin F, et al. Role of Polymer Micelles in the Delivery of Photodynamic Therapy Agent to Liposomes and Cells. Cancers. févr 2020;12(2):384.

Dynamic dysregulation of Tenascin-X could lead to tumoral cell proliferation during pancreatic carcinogenesis

Céline Schmitter[1*], Sophie Liot[1], Luna Gonzalez[1], Perrine Mercier-Gouy[1], Valérie Hervieu[2], Manuel Koch[3], Ana Hennino[4], Sylvie Ricard-Blum[5], Ulrich Valcourt[1], Elise Lambert[1]

[1] *Laboratoire de Biologie Tissulaire et Ingénierie Thérapeutique (LBTI), UMR CNRS 5305, Université Claude Bernard Lyon 1, Institut de Biologie et Chimie des Protéines, Lyon, France*

[2] *Service d'Anatomopathologie, Groupement Hospitalier Est, Hospices Civils de Lyon, Lyon, France*

[3] *Institute for Dental Research and Oral Musculoskeletal Biology, Center for Biochemistry, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany*

[4] *Centre de Recherche en Cancérologie de Lyon (CRCL), Centre Léon Bérard, INSERM 1052, CNRS 5286, Université de Lyon, Université Claude Bernard Lyon 1, Lyon, France*

[5] *Université Claude Bernard Lyon 1, CNRS, INSA Lyon, CPE, Institute of Molecular and Supramolecular Chemistry and Biochemistry, UMR 5246, Villeurbanne, France*

* celine.schmitter@univ-lyon1.fr

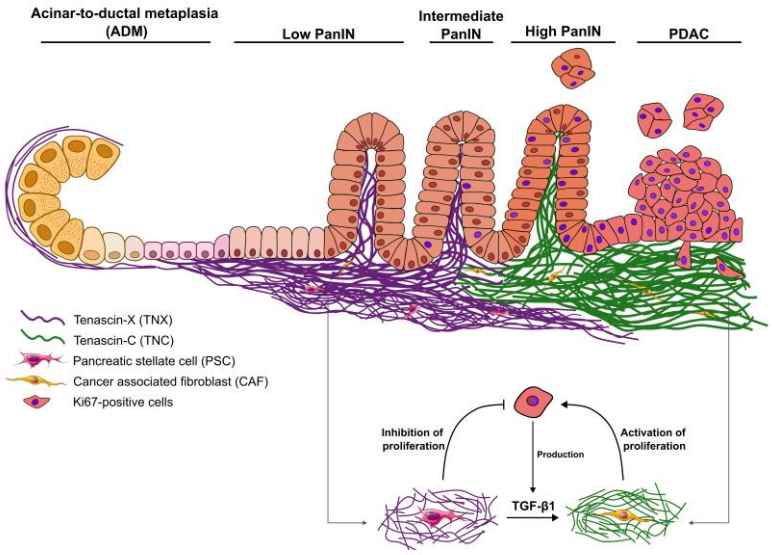
Keywords: Pancreatic cancer, Tenascin-X, CRISPR activator strategy, Proliferation

Abstract:

Throughout Pancreatic Ductal AdenoCarcinoma (PDAC) progression, a dense extracellular matrix is deposited around neoplastic cells and accompanies tumor development and aggressiveness. This stroma could harbor targets for innovative therapies. We investigated, in PDAC, the expression and role of Tenascin-X (TNX), a matricellular protein that we previously described as commonly lost in numerous solid tumors.

In contrast to TNC, we demonstrated that *TNXB* gene expression and TNX protein level are drastically decreased in human PDAC samples. This loss correlates with reduced patient survival and could be caused by TGF-beta based on in-vitro experiments. Using various mouse models, we demonstrated that TNX is first heavily deposited around low-grade lesions before being decreased in later stages suggesting an elaborate stromal reaction in the course of PDAC development. Interestingly, in human and mouse PDAC samples, we correlated low TNX level with increased cell proliferation (**Liot, Schmitter *et al.* 2025, preprint**). Therefore, to further investigate the role of TNX on tumor cells, we implemented a CRISPR activator (CRISPRa) technique to restore TNX production in immortalized Cancer-Associated Fibroblasts (CAFs) extracted from a patient suffering from PDAC and that do not express *TNXB* anymore. *In vitro* 2D and 3D models revealed that the CAFs modified by CRISPRa produce and release more TNX in their microenvironment and that the TNX seems to integrate into the ECM network. The impact of this newly formed TNX-rich matrix on the behavior of pancreatic cancer cells is currently being studied in the laboratory.

Our results highlight the complex modulation of TNX deposition throughout PDAC development and suggest TNX as an anti-proliferative protein thus rending this protein a valuable target for the development of new drugs for PDAC and/or solid tumor treatment.



Graphical abstract: TNXB regulation throughout PDAC development.

References:

[1]: S. Liot; C. Schmitter; P. Mercier-Gouy; N. El Kholti; J. Balas; L. Prigent; A. Aubert; C. Lethias; J.L. Chitty; M. Koch; T. Fenouil; T.R. Cox; A. Hennino; V. Hervieu; S. Ricard-Blum; U. Valcourt; E. Lambert. *BioRxiv*, 2025, DOI: http://dx.doi.org/10.1101/2025.02.07.635757

Impact of type I collagen carbamylation on the phenotypic modulation of vascular smooth muscle cells

CADORET Lucile [a*], OKWIEKA Anaïs [a], DOUÉ Manon [a], BERQUAND Alexandre [a], PIETREMENT Christine [a], GILLERY Philippe [a], JAISSON Stéphane [a]

[a] *CNRS/URCA Joint Research Unit 7369 - MEDyC (Extracellular Matrix and Cellular Dynamics) / Team 2: Matrix Aging and Vascular Remodeling, REIMS.*

* lucile.cadoret@univ-reims.fr

Keywords: Collagen – Smooth Muscle Cells – Carbamylation – Atherosclerosis

Abstract:

Cardiovascular diseases are the leading cause of death worldwide and represent a major public health challenge. Understanding the mechanisms involved in their progression, as well as identifying new therapeutic strategies, is therefore essential. Among the emerging avenues, protein molecular aging—which includes non-enzymatic modifications that progressively impair structural and functional properties—appears to play a key role. One such modification, carbamylation, results from the irreversible binding of isocyanic acid—mainly derived from urea dissociation—to protein amino groups.

This study investigates whether type I collagen carbamylation, present in the vascular media, can induce a phenotypic transition in vascular smooth muscle cells (VSMCs), promoting a shift from a contractile to a synthetic phenotype, thereby contributing to cardiovascular pathologies.

In this in vitro study, we cultured MOVAS cells (a mouse aortic VSMC line) and primary VSMCs isolated from C57Bl/6 mouse aortas on a type I collagen matrix extracted from rat tail tendons for 48 hours. Collagen carbamylation was induced by incubation with sodium cyanate (0.1 M, 24 h, 37°C) and confirmed by quantification of homocitrulline, a specific marker of carbamylation.

We first observed a marked morphological change in VSMCs exposed to carbamylated collagen, adopting a more complex structure. This phenotypic switch was further assessed at the transcriptomic level using contractile markers (*Acta2, Cnn1, Myocd*) and synthetic markers (*Lgals3, Klf4*). Results showed that prolonged exposure to modified collagen promotes VSMC dedifferentiation toward a synthetic phenotype, with a 40% increase in synthetic markers and a 50% decrease in contractile markers.

Atomic force microscopy analysis also revealed a significant reduction in cell stiffness, with a 70% decrease in Young's modulus. While VSMC proliferation was not affected, cell adhesion and migration were significantly increased in response to carbamylated collagen.

These findings suggest that collagen carbamylation may play a key role in vascular remodeling associated with cardiovascular diseases by modulating VSMC phenotype.

To assess these effects in vivo, we studied atherosclerotic plaque development in ApoE$^{-/-}$ mice, a model that spontaneously develops atherosclerosis. Matrix protein carbamylation was induced in some mice by adding sodium cyanate to their drinking water. Disease severity was assessed through histological staining (H&E, Sirius Red, Van Kossa), immunofluorescence for VSMC evaluation, and HREM-Histo3D imaging.

These results support the hypothesis that matrix protein carbamylation may play a critical role in the development and progression of atherosclerosis.

Loss of function variants in *ADAMTS6*: Connective tissue, Heart defect, thoracic Aortic aneurysm and Neuro developmental Syndrome (CHANS)

Huguet Herrero Júlia[1]*, Arnaud Pauline[1, 2, 3]*, Bibimbou Angelique[1], Seifert Deborah E.[4], Mougin Zakaria[1], Benarroch Louise[1, 15], Michot Caroline[7], Capri Yline[8], Ruaud Lyse[8], Dupuis-Girod Sophie[9], Guilhem Alexandre[9], Beyer Constance[10], Bajolle Fanny[11], Milleron Oliver[1, 3], Jondeau Guillaume[1, 3], Hanna Nadine[1, 2], Reinhardt Dieter P.[12,13], Guo Dong-Chuan[14], Milewicz Dianna[14], Boileau Catherine[1, 2], Aubart Mélodie[6], Mead Timothy J.[4,5], Le Goff Carine[1°]

[1] *Université Paris Cité et Université Sorbonne Paris nord, INSERM U1148, LVTS, Paris, France;*
[2] *Département de Génétique, AP-HP, Bichat Hospital, Paris, France;*
[3] *National Reference Centre for Marfan Syndrome and related disorders, AP-HP, Bichat Hospital, Paris, France;*
[4] *Department of Pediatrics, Case Western Reserve University, Cleveland, OH, USA*
[5] *University Hospitals Rainbow Babies and Children's Hospital, Cleveland, OH, USA*
[6] *Service de Neuropédiatrie, AP-HP, Hôpital Necker-Enfants Malades, Paris, France;*
[7] *Service de Génétique, AP-HP, Hôpital Necker-Enfants Malades, Paris, France;*
[8] *Service de Génétique, AP-HP, Hôpital Robert Debré, Paris, France;*
[9] *Service de Génétique, Hospices Civils de Lyon, Bron, France;*
[10] *Service de Cardiologie, AP-HP, Hôpital Robert Debré, Paris, France;*
[11] *Service de Cardiologie, AP-HP, Hôpital Necker-Enfants Malades, Paris, France;*
[12] *Faculty of Medicine and Health Sciences, Department of Anatomy and Cell Biology, McGill University, Montréal, QC, Canada*
[13] *Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montréal, Canada*
[14] *University of Texas Health Science Center at Houston, Houston, TX, USA*
[15] *Sorbonne Université, Inserm, Institut de Myologie, Centre de Recherche en myologie, Paris, France*

° carine.le-goff@inserm.fr

Keywords: aorta; ADAMTS; fibrillin; aneurysm; new syndrome

Abstract:
Marfan syndrome (MS), Loeys-Dietz syndrome (LDS), and heritable thoracic aortic aneurysms and dissections (hTAAD) are autosomal dominant connective tissue disorders with overlapping clinical features and underlying molecular heterogeneity. While most cases are explained by pathogenic variants in genes involved in extracellular matrix structure or TGFβ signaling, a large proportion of hTAAD cases remain idiopathic. Through exome and genome sequencing in a French diagnostic cohort, we identified rare deleterious variants in *ADAMTS6* in four unrelated individuals with syndromic or isolated vascular disease. Functional studies demonstrated that these variants impair *ADAMTS6* secretion or function, particularly in processing fibrillin-1 (FBN1) and fibrillin-2 (FBN2), resulting in extracellular matrix accumulation and microfibril disorganization. One variant, p.(Leu814Arg), further disrupted the Hippo and TGFβ signaling pathways and altered cell adhesion. Analysis of a patient-derived fibroblast model and *Adamts6*-deficient mice supported a pathogenic role for *ADAMTS6* loss-of-function in a novel connective tissue disorder. Clinical phenotypes spanned from early-onset syndromic presentations with cardiovascular, craniofacial, skeletal, and neurodevelopmental involvement to isolated adult-onset hTAAD. We propose *ADAMTS6* deficiency defines a new connective tissue disorder, termed CHANS (Connective tissue, Heart defect, thoracic Aortic aneurysm, and Neurodevelopmental Syndrome), expanding the spectrum of ADAMTS-related pathologies and highlighting its key role in vascular and ECM homeostasis.

Title : Senescence-Driven Changes in Heparan Sulfate and Proteoglycan Structure and Expression in Osteoarthritis

Amina Boukhobza*[a], Benjamin Even*[a], Camille Flageollet[a], Florent Eymard1[a, b], Patricia Albanese*[a]

[a] *Gly-CRRET EA 4397 UPEC FST, Créteil, France*
[b] *AP-HP Hôpital Henri Mondor, Créteil, France*

* amina.boukhobza@u-pec.fr, benjamin.even@u-pec.fr, albanese@u-pec.fr

Keywords: Heparan Sulfates, Senescence, Osteoarthritis, Inflammation.

Abstract:

Osteoarthritis (OA) is an aging associated disease and is characterized by an important degradation of the extracellular matrix (ECM) components of cartilage, such as proteoglycans (PG) and glycosaminoglycans (GAG), whose degradation products contribute to inflammation of the synovial membrane[1]. Among GAG, we focus on **heparan sulfates** (HS), as alterations in their expression, structure, and function have been observed in OA notably in cartilage [2] and in synovial fluid and cells (unpublished data). These changes are particularly relevant given that HS can interact with a wide range of heparin-binding proteins (HBPs) through specific sulfation patterns, enabling them to modulate key signaling pathways involved in joint pathophysiological processes. While recent studies have shown that 1) **senescence** contributes to the pathophysiology of OA[3] and 2) **HS proteoglycans** (HSPG) can modulate cellular senescence in various biological contexts[4], the potential interplay between HSPG and senescence in OA remains unexplored. Moreover, different inflammatory phenotypes and HS profiles are reported in synovial tissue[5]. In this context, our aim is to characterize the expression and structure alteration profile **of HS during senescence** of synoviocytes from OA patients, and to make a link with inflammatory signalling that pave the way of OA**.**

We developed and validated a senescence model induced by H_2O_2-mediated oxidative stress in synoviocytes derived from the synovial membranes of OA patients. Immunofluorescence staining revealed an increase in HS levels and alterations in N, 2-O and 3-O sulfation profiles during senescence. Furthermore, the induction of senescence appeared to vary according to the inflammatory status of the synovial membranes and synoviocytes. In parallel, gene expression analysis of enzymes involved in HS biosynthesis revealed modulation of specific HS sulfotransferases involved in the specific patterns observed by immunofluorescence staining. Notably, 3-O-sulfatransferase expression, which is responsible for the 3-O-sulfation of HS chains, differs between synoviocytes derived from non-inflamed and inflamed synovial tissue after senescence induction.

These findings suggest that senescence induction is associated with the modifications of the HS sulfatation profile and inflammatory status in OA synoviocytes. A more precise structural characterization of HS sulfation profiles, HSPG and expression patterns of HS biosynthetic enzymes is currently ongoing. Functional analysis of overexpression or inactivation of one of the identified glycanic targets should allow us to determine whether HS act as active mediators or as biomarkers of senescence in OA.

References:

[1]: Mobasheri A, Rayman MP, Gualillo O, Sellam J, van der Kraan P, Fearon U. The role of metabolism in the pathogenesis of osteoarthritis. *Nat Rev Rheumatol*. 2017 May;**13(5)**:302-311.
[2]: Shamdani S, Chantepie S, Flageollet C, Henni-Chebra N, Jouan Y, Eymard F, Hay E, Cohen-Solal M, Papy-Garcia D, Chevalier X, Albanese P. Heparan sulfate functions are altered in the osteoarthritic cartilage. *Arthritis Res Ther*. 2020 Dec 7;**22(1)**:283.
[3]: Kang D, Jung SH, Lee GH, Lee S, Park HJ, Ko YG, Kim YN, Lee JS. Sulfated syndecan 1 is critical to preventing cellular senescence by modulating fibroblast growth factor receptor endocytosis. *FASEB J*. 2020 Aug;**34(8)**:10316-10328.
[4]: Herath D, Even B, Oranger M, Foresti R, Papy D, Boyer L, Boczkowski J, Dagouassat M. Secreted phospholipase A2 XIIA triggers a mitochondrial damage-induced senescence in chronic obstructive pulmonary disease fibroblasts. *Free Radic Biol Med.* 2023 Aug 20; **205**:129-140
[5]: Deligne C, Casulli S, Pigenet A, Bougault C, Campillo-Gimenez L, Nourissat G, Berenbaum F, Elbim C, Houard X. Differential expression of interleukin-17 and interleukin-22 in inflamed and non-inflamed synovium from osteoarthritis patients. *Osteoarthritis Cartilage*. 2015 Nov;**23(11)**:1843-52.

Posters

Extracellular vesicles derived from ovarian cancer cell lines discriminated by biochemical and FTIR spectroscopy approaches

Lefkothea Pantazi[a,b],Valérie Untereiner[c], Paolo Rosales[a,d], Romain Rivet[a], Sandra Audonnet[e], Isabelle Proult[a], Laurent Ramont[a,f], Ganesh D. Sockalingum[g], and Stéphane Brézillon*[a]

[a] *Université de Reims Champagne-Ardenne, UMR CNRS 7369, MEDyC, Reims, France*
[b] *University of Patras, Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, Patras, Greece*
[c] *Université de Reims Champagne-Ardenne, URCATech, PICT, Reims, France*
[d] *UNNOBA/CIT NOBA (UNNOBA-UNSADA-CONICET), Laboratorio de Microambiente Tumoral, CIBA, Junín, Argentina*
[e] *Université de Reims Champagne-Ardenne, URCATech, URCACyt, Reims, France*
[f] *CHU de Reims, Service Biochimie-Pharmacologie-Toxicologie, Reims, France*
[g] *Université de Reims Champagne-Ardenne, UR7506, BioSpecT, Reims, France*

* stephane.brezillon@univ-reims.fr

Keywords: Extracellular Vesicles, Ovarian cancer cell lines, FTIR spectroscopy, Chemometrics

Abstract:

Ovarian cancer is the most lethal cancer among gynaecological malignancies. Due to the lack of early symptoms and screening tools, patients are diagnosed in advanced stages. Cancer invasion and metastasis through the extracellular matrix (ECM) are enhanced by tumour cell Extracellular Vesicles (EV). The aim of this study was to characterise the EVs derived from two ovarian cancer cell lines (ES2 and SKOV3) using biochemical and vibrational spectroscopic approaches. EVs were prepared by ultracentrifugation and characterised by Nanoparticle Tracking Analysis. Their surface proteins were assessed by MACSPlex EV kit for human exosomes. The presence of MMP14 and integrin subunits was evaluated in EVs and cell protein extracts by Western immunoblotting. Both EVs and cells were measured by Fourier transform infrared spectroscopy (FTIR) and data were analysed by hierarchical cluster analysis (HCA). Spectral differences were observed in the lipids and polysaccharides regions, both between the SKOV3 and ES2 cells and their corresponding EVs, which allowed a good delineation by HCA. The differences in the biochemical data were confirmed by similar and specific features exhibited in their respective infrared spectral signatures. ES2 EVs exhibited an enrichment in MMP14 in agreement with the aggressiveness of this ovarian cancer metastatic cell line.

The funding from the Ligue Contre le Cancer (CCIR-GE) is gratefully acknowledged. L. Pantazi was an Erasmus+ Master student and P. Rosales was funded by the Horizon Europe Hepinib Programme. This project has received funding from the European Union's Horizon Europe programme Staff Exchanges under the Marie Sklodowska-Curie grant agreement No. 101086322. The authors thank the URCATech, PICT-IBiSA and URCACyt platforms of the University of Reims Champagne-Ardenne for instrument facilities.

Reference:

L. Pantazi, V. Untereiner, P. Rosales, R. Rivet, S. Audonnet, I. Proult, L. Ramont, G.D. Sockalingum, S. Brézillon. Extracellular vesicles derived from ovarian cancer cell lines discriminated by biochemical and Fourier transform infrared spectroscopy approaches. Analyst. 2025; 150(7):1280-1292.

FTIR and biochemical characterisation of glycosaminoglycans (GAGs) content in ovarian cancer cells and their secretome

Elise Vincent[a,b], Valérie Untereiner[c], Romain Rivet[a], Isabelle Proult[a], Ganesh D Sockalingum[b] Stéphane Brézillon[a*]

[a]*Université de Reims Champagne-Ardenne, CNRS UMR 7369, MEDyC, Reims, France.*
[b]*Université de Reims Champagne-Ardenne, UR7506, BioSpecT, Reims, France.*
[c]*Université de Reims Champagne-Ardenne, URCATech, PICT, Reims, France.*

* stephane.brezillon@univ-reims.fr

Keywords: FTIR spectroscopy, Glycosaminoglycans, Ovarian cancer cells, Secretome,

Abstract:

Ovarian cancer remains one of the deadliest cancers due to the lack of robust screening methods for early detection. During tumour progression, the extracellular matrix (ECM) is altered and associated with the disorganisation of macromolecules such as collagens and proteoglycans (proteins covalently linked with sulphated glycosaminoglycans (GAGs)). GAGs are polysaccharides characterised by repeating disaccharide units and are involved in the sequestration of growth factors regulating tumour progression. In ovarian cancer, a high expression of chondroitin sulphate has been described (ten Dam GB *et al*., 2007). Thus, GAGs characterisation at the cell level and their secreted GAGs (secretome) is of great interest. In this study, FTIR spectroscopy was used to characterise ovarian cancer cell lines (synthesising GAGs) and their secretome.

Three ovarian cancer cell lines (SKOV3, CAOV3, OVCAR3), two Chinese hamster ovary cells (CHO-K1, pgsA-745) and their secretome were analysed. Secreted sulphated GAGs were quantified using the specific Blyscan® test. In parallel, the secretome was measured using high throughput FTIR spectroscopy

(INVENIO S, Bruker Optic). Solutions containing one microgram of sulphated GAGs were deposited on 384-well silicon plate and dried at room temperature. Cells were grown overnight on calcium fluoride windows at $1.5x10^4$ cells/mL and analysed, after fixation in 4% paraformaldehyde, at the single cell level using the Spotlight 400 (Perkin Elmer) FTIR microscope. Both secretome and cell FTIR data were obtained in the transmission mode, pre-processed in the same manner (baseline correction and vector normalisation) and analysed by hierarchical cluster analysis (HCA).

After normalising to the protein content, Blyscan® results showed a difference in the capacity of the cells to secrete sulphated GAGs with the following tendency: CHO-K1 > SKOV3 > OVCAR3 > pgsA-745 > CAOV3 with a tenfold increase between CHO-K1 and CAOV3. The cell secretome FTIR spectra revealed specific signatures associated to GAG molecules, in particular the sulphate and polysaccharides absorptions (1300-900 cm^{-1}). HCA analysis of secretome reveals a first group containing CHO-K1 and pgsA-745 cells representing control cells differing only by their capacity of GAG synthesis. The second cluster groups together the ovarian cancer cells SKOV3, OVCAR3 while CAOV3 appears very distinct from the two groups. At the single cell level, a similar grouping of the CHO cells was observed but not for the ovarian cancer cells.

The secretome biochemical analysis indicates that SKOV3 and OVCAR3 exhibit comparable amounts of sulphated GAGs in comparison with CHO-K1 and in contrast with pgsA-745 and CAOV3. These results suggest a strong variability in the sulphated GAGs content between ovarian cancer cell lines independently of their phenotype. A good correlation is obtained between biochemical assay and secretome FTIR data. At the single-cell level, it is again observed that CHO-K1 and pgsA-745 form one cluster in contrast to ovarian cancer cell lines. However, the correlation with biochemical data is less evident. Overall, this study shows an interesting approach to investigate the GAG synthesised and secreted by ovarian cancer cells. This could be helpful for implementing diagnostic and therapeutic strategies in the treatment of this disease.

Funding from ANR 20-CE44-0018 GLYCOTARGET is acknowledged. The authors thank the URCATech, PICT-IBiSA platform of the University of Reims Champagne-Ardenne for instrument facilities.

Reference:

ten Dam GB et al., Antibody GD3G7 selected against embryonic glycosaminoglycans defines chondroitin sulfate-E domains highly up-regulated in ovarian cancer and involved in vascular endothelial growth factor binding. Am J Pathol. 2007 Oct;171(4):1324-33.

FTIR characterisation of chondroitin sulfate E (CS-E) di-, tetra-, and hexasaccharide derivatives and their biotinylated or reducing conjugates

Elise Vincent[a,b], Valérie Untereiner[c], Florian Chabot[d], Aude Vibert[d], Marie Schuler[d], Jorgan Guard[a,b], Romain Rivet[b], Isabelle Proult[b], Chrystel Lopin-Bon[d], Stéphane Brézillon[*b], Ganesh D Sockalingum[a]

[a]*Université de Reims Champagne-Ardenne, UR7506, BioSpecT, Reims, France.*
[b]*Université de Reims Champagne-Ardenne, CNRS UMR 7369, MEDyC, Reims, France.*
[c]*Université de Reims Champagne-Ardenne, URCATech, PICT, Reims, France.*
[d]*Institut de Chimie Organique et Analytique (ICOA), UMR 7311 CNRS et Université d'Orléans, Orléans, France.*

* stephane.brezillon@univ-reims.fr

Keywords: FTIR spectroscopy, oligosaccharides, chondroitin sulfate, unsupervised classification

Abstract:
Sulfated glycosaminoglycans (GAGs), namely chondroitin sulfate (CS), dermatan sulfate, keratan sulfate, heparin, and heparan sulfate are linear complex polysaccharides covalently attached to core proteins to form proteoglycans. They are present at the cell surface and in the extracellular matrix and play a key role in the regulation of cellular microenvironmental effectors. To better understand the biological functions of GAGs and particularly of CS-E (4,6-disulfated) at the molecular level, structurally well-defined oligosaccharides are necessary. Chemically synthesized biotinylated conjugates are useful to study the interaction with proteins at both the intra- and extracellular level. Here, FTIR spectroscopy was used to characterize nine chondroitin oligosaccharides that included biotinylated or reducing CS-E di-, tetra- and hexasaccharides as well as their non-sulfated analogs. Spectral features characteristic of oligosaccharide (1640, 1626, 1565, 1418, 1375, 1160 cm^{-1}), CS-E (1280-1200, 1134, 1065, 1034, 1000, 927, 866-860, 815 cm^{-1}), and biotin (1681, 1460, 1425, 792 cm^{-1}) vibrational modes were identified. FTIR spectroscopy was sensitive enough to reveal structural microheterogeneity allowing distinguishing C-4 and C-6 sulfated isoforms. CS-E and biotin specific signatures were obtained by difference spectra. The PCA plots revealed three distinct groups: biotinylated oligosaccharides, CS-E biotinylated oligosaccharides and CS-E reducing oligosaccharides. Furthermore, the first component, clearly distinguished sulfated from non-sulfated forms whereas component two tended to discriminate according to the chain length exclusively for the non sulfated oligosaccharides. Identifying the spectral signatures of these oligosaccharides is an important step for future research on the monitoring of the internalisation of the oligosaccharides and cell penetrating peptides bound forms in drug delivery studies.

Funding from ANR 20-CE44-0018 GLYCOTARGET is acknowledged. The authors thank the URCATech, PICT-IBiSA platform of the University of Reims Champagne-Ardenne for instrument facilities.

Reference:
E. Vincent, V. Untereiner, F. Chabot, A. Vibert, M. Schuler, J. Guard, R. Rivet, I. Proult, C. Lopin-Bon, S. Brézillon, G.D. Sockalingum. FTIR characterisation of chondroitin sulfate E (CS-E) di-, tetra-, and hexasaccharide derivatives and their biotinylated or reducing conjugates. Accepted for publication in Analyst, 2025.

Effects of tattoo ink in combination with electroporation in engineered dermal sheets

Ophélie Cordier [a], Emma Barrère [a], Nicolas Mattei [a], Caroline Ladurantie [a], Marie-Pierre Rols [a], Muriel Golzio [a], Jelena Kolosnjaj-Tabi *[a]

[a] *Institute of Pharmacology and Structural Biology, 205 route de Narbonne, 31077 Toulouse, FRANCE (CNRS UMR 5089)*

* Jelena.Kolosnjaj-Tabi@ipbs.fr

Keywords: electroporation; tattoo ink; nanoparticles; engineered dermal sheet

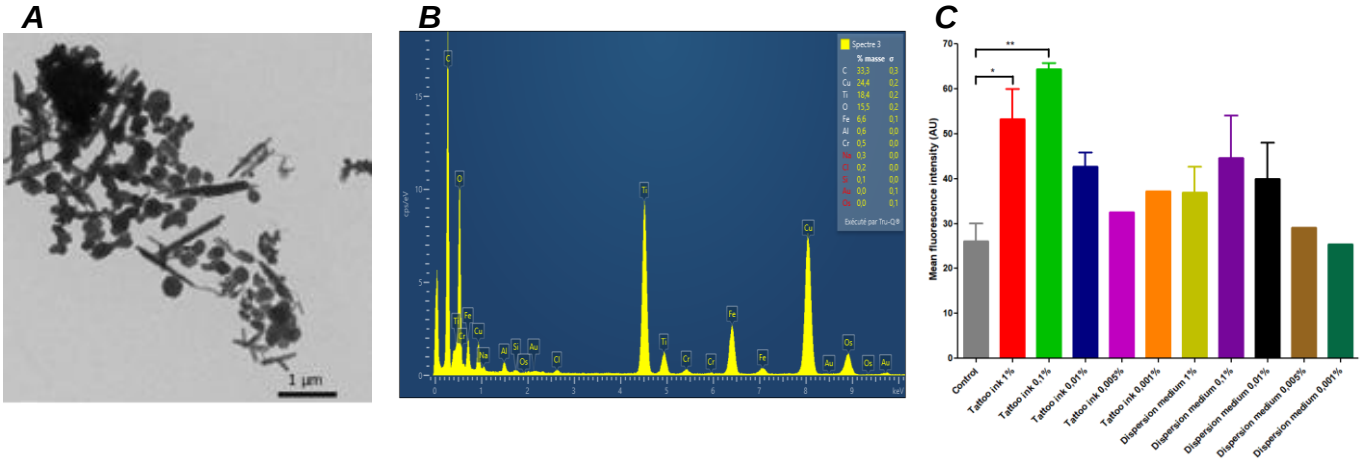
Abstract:

Electroporation is a technique that exploits electrical pulses to increase cell membrane permeability, allowing anticancer agents to penetrate tumor cells or allowing direct non-thermal cancer cell ablation. The approach combining chemotherapy and electric pulses is known as electrochemotherapy (ECT). This method is clinically used to treat tumors, including skin cancers such as melanoma [1]. In parallel, the prevalence of tattoos is rising, both for aesthetic and reparative purposes. Tattoo inks contain micro- and nanoparticles (NPs), notably titanium dioxide and iron oxides, which serve as pigments. During ECT, these particles embedded in the skin might interact with electric fields, potentially altering or amplifying the effects of electroporation [2].

Our study investigates the combined biological effects of these nanoparticles and electroporation using a three-dimensional engineered dermal sheet model. The tattoo ink was observed by transmission electron microscopy (TEM) (Fig. A) and characterized by energy-dispersive X-ray spectroscopy (EDX) (Fig. B). Control (ink or surfactant-free) dermal sheets and dermal sheets incubated for two days either with tattoo-ink containing metal/metal oxide particles (Fig C), or only the surfactant used to formulate the tattoo-ink suspension were studied. The models underwent electroporation (8 pulses, 100 µs duration, 1 Hz pulse repetition rate, at the electric field intensity of 1000 V/cm) and non-pulsed dermal sheets were used as controls. Propidium iodide was used as cell membrane permeabilization probe.

Our results show that the presence of tattoo ink-derived nanoparticles significantly increases dermal sheet permeabilization, while dermal sheets exposed to nanoparticle-free surfactant used for tattoo ink dispersion showed only a slight increase in permeability, likely due to pulse-induced cellular permeabilization in combination with the surfactants present in the dispersion medium.

The results of our study show that tattoo-derived nanoparticles may alter the permeability of cells constituting the dermal sheets. Further studies are planned to determine whether, and to what extent, the extracellular matrix adjacent to tattoo inks might be affected by electric pulses, and what implication might it have in cutaneous cancer treatment.



A: TEM micrograph of tattoo ink suspension. B: EDX of tattoo ink suspension. C: Mean fluorescence intensity following the permeabilization of dermal sheets after exposure to pulsed electric fields at 1000 V/cm.

References:

[1] Bastrup FA, Vissing M, Gehl J. Electrochemotherapy for metastatic cutaneous melanoma. Acta Oncol. 2022 May ; 61(5):531-532. doi: 10.1080/0284186X.2022.2057199

[2] Lekner J. Electroporation in cancer therapy without insertion of electrodes. Phys Med Biol. 2014 Oct 21;59(20):6031-42

Overcoming T cell exclusion in PDAC: Periostin as a therapeutic target?

Cédric FABRE [a,b], Petnoi PETSOPHONSAKUL [a], Solène DEUTSCH [a], Corinne BOUSQUET [a], Jérome CROS [c], Rémy NICOLLE [c], Frédéric LAGARRIGUE [b], Christine JEAN [a*]

[a] *Centre de Recherche en Cancérologie de Toulouse – Team MICROPANC, UMR1037, Toulouse, France*
[b] *Institut de Pharmacologie et de Biologie Structurale – Team Integrins in Immune Cells, UMR5089, Toulouse, France*
[c] *Centre de Recherche sur l'Inflammation, UMR1149, Paris, France*

* christine.jean@inserm.fr

Keywords: Pancreatic cancer biology, Stroma, Antitumor immunity, Extracellular matrix

Abstract:

Pancreatic Ductal Adenocarcinoma (PDAC) is one of the deadliest cancer worldwide, and new treatment are urgently needed. This solid tumor is characterized on the one hand by a highly desmoplastic stroma, and on the other hand by a poor and heterogeneous infiltration of CD8+ T cells. We hypothesize that T cell exclusion far from tumor cells, thus preventing physical contact that trigger an antitumor response, rely on extracellular matrix (ECM) organization and structure. Characterizing the mechanisms involved in such an interaction between the ECM and T cells should allow the identification of targets in order to favor T cell infiltration and subsequently restore the antitumor immune response in PDAC.

Using microdissection and transcriptomic analysis of various human tumor areas, we identified ECM proteins positively or negatively associated with $CD8^+$ T cell infiltration. These ECM proteins have been studied by multiplex imaging and the glycoprotein periostin emerged as an interesting candidate. Indeed, while periostin-deposition is highly heterogeneous, periostin-rich tumor regions are consistently devoided of $CD8^+$ T cells in human PDAC samples.

We then looked for the periostin origin using public PDAC single-cell RNA sequencing database and identified the Pancreatic Stellate Cells (PSCs) and the Cancer-Associated Fibroblasts (CAFs) as the main periostin producers. To better understand the potential role of the periostin in the T cell exclusion phenotype, we generated an orthotopic and syngenic co-graft model of tumor cells with PSCs. Echoing the human disease, our mouse model revealed a significant segregation: periostin and $CD8^+$ T cells are mutually excluded.

In parallel, we questioned the T cell ECM receptor repertoire using human PDAC scRNAseq database and found out that the expression of the main periostin receptor, the αV integrin, is significantly decreased in PDAC infiltrating T cells compared to the one from healthy pancreas, being consistent with our observations.

Future work will assess whether periostin depletion in PSCs restores immune infiltration through co-grafting experiments and spatial multiplex imaging. Ultimately, we can anticipate that such combination with immune checkpoint blockade, while targeting periostin-mediated lymphocyte exclusion, may enhance antitumor immune response in PDAC.

Searching for Tumor-Specific Matrisome Markers in Endometrial Cancer: a new role for Annexins in tumor growth and metastasis.

Tom ROUSSET[a], Marie DAUTREMONT[a,b], Gaëtan THIVOLLE LIOUX[a,b], Patrick MEHLEN[a,c,d], Laurent FATTET*[a]

[a] *Apoptosis, Cancer and Development laboratory, Cancer Research Center of Lyon (CRCL), Lyon, FRANCE*
[b] *Claude Bernard University Lyon 1, Lyon, France*
[c] *Leon Berard Comprehensive Cancer Center, Lyon, FRANCE*
[d] *Netris Pharma, Lyon, FRANCE*

* laurent.fattet@lyon.unicancer.fr

Keywords: Annexins, Matrisome, Endometrial cancer, Metastasis

Abstract:

The extracellular matrix (ECM) has long been considered as an inert scaffold holding a tissue together but is now regarded as an important regulator of cell behavior and tissue function, both in physiological and pathological contexts. The ECM belongs to a complex network of more than 1000 Matrisome proteins with structural roles (collagens, glycoproteins, proteoglycans) but also regulatory functions, as harbored by secreted factors, ECM regulators and ECM-affiliated proteins[1].

Solid tumor development and metastatic progression has mainly focused on biochemical signals regulating downstream signaling pathways. The power of the extracellular compartment, and especially the non-cellular fraction, has only been recently appreciated and it is now admitted that mechanical cues from the ECM, and overall tissue stiffness, can directly affect cell behavior, such as the epithelial-to-mesenchymal transition (EMT) and invasion via various mechanotransduction pathways[2-5].

This project aims at identifying novel potential target for therapy within the Matrisome, called Tumor-Specific Matrisome Markers (TSMMs), of various tumor models, including a syngeneic endometrial cancer orthotopic xenograft model named MECPK (Mouse Endometrial Cancer PTEN-deleted and Kras-activated)[6]. To begin with, we performed mass spectrometry analyses (LC-MS/MS) of decellularized endometrial tumors samples together with paired healthy tissues and identified promising candidates within the Matrisome that are currently being studied. In particular, a member of the Annexin family is presented here in a proof-of-concept study showing that a TSMM, found highly expressed in tumor ECMs and absent from normal tissue, shows significant effect on tumor growth and metastatic progression *in vivo*. Single cell RNA sequencing data shows a restricted expression of this Annexin by a sub-population of tumor cells enriched for the ECM production/basement membrane signature. Interestingly, modulating the expression of this candidate *in vitro* does not affect tumor cell proliferation, migration or apoptosis, suggesting unique tumor-promoting functions in the tumor microenvironment. We are now investigating by which mechanism this tumor cell-derived matrisome extracellular protein can regulate endometrial tumor growth and/or tumor metastasis.

Our long-term goal is to find new actionable targets in the tumor microenvironment and promote the development of novel molecular imaging theragnostic approach, as well as vectorized radiotherapy-based therapeutic strategies.

References:

[1]: A. Naba; K.R. Clauser; …; and R.O. Hynes, *Mol Cell Proteomics*, 2012, **11,** M111.014647.
[2]: K.R. Levental; H. Yu; …; and V.M. Weaver, *Cell,* 2009, **139**, 891–906.
[3]: M.J. Paszek; N. Zahir; …; and V.M. Weaver, *Cancer Cell*, 2005, **8**, 241–54.
[4]: S.W. Wei; L. Fattet; …; and J. Yang, *Nat Cell Biol*, 2015, **17**, 678-88.
[5]: L. Fattet; H.Y. Jung; …; and J. Yang, *Dev Cell*, 2020, **54**, 302-316.e7.
[6]: A.M. Fedorko; T.H. Kim; …; J.I. Risinger, *Heliyon*, 2020, **6**, e04075.

Modification of the vascular extracellular matrix in Marfan syndrome: regulation of the inflammatory response and influence of elastin peptides on autophagy

Sabrina Haddou *[a], Alexandre Berquand [a], Hervé Sartelet [a], Amar Bennasroune [a], Sébastien Blaise [a], Pascal Maurice [a†], Rida Al-Rifai [b,c], Luna Chetrit [b,c], Carine Le Goff [d], Hafid Ait-Oufella [b,c,e], Béatrice Romier-Crouzet [a], Laurent Duca [a]

[a] *UMR CNRS 7369 MEDyC, Université de Reims Champagne-Ardenne, Reims, France*
[b] *Inserm U970 Paris Centre de Recherche Cardiovasculaire (PARCC), Paris, France*
[c] *POLYGON therapeutics, Levallois Perret, France*
[d] *INSERM U1148, LVTS Université Paris Cité et Université Sorbonne Paris Nord*
[e] *Medical Intensive Care Unit, Hôpital Saint-Antoine, AP-HP, Sorbonne Université, Paris, France*

* *sabrina.haddou@univ-reims.fr*

Keywords: Marfan syndrome; inflammation; autophagy; elastin-derived peptides; vascular remodeling; biomarkers

Abstract:

Marfan syndrome is caused by mutations in the gene encoding fibrillin-1. Clinical manifestations include abnormally long limbs and fingers, joint hyperlaxity, as well as pulmonary and cardiovascular complications, the latter being associated with high mortality. Currently, no curative treatment is available for Marfan syndrome, as the underlying mechanisms remain poorly understood, although inflammatory processes and alterations in autophagic flux in vascular smooth muscle cells (VSMCs) are thought to contribute to its aortic manifestations.

The aim of this study was to investigate the role of the anti-inflammatory receptor TREM2 in vascular and extracellular matrix remodeling in Marfan syndrome, as well as the impact of elastolysis on VSMC autophagic flux.

We demonstrate that the absence of TREM2 in mice induces arterial dilation associated with pronounced extracellular matrix remodeling, revealed by Second Harmonic Generation (SHG) imaging. Mechanical analysis by Atomic Force Microscopy (AFM) further highlights altered fiber elasticity, confirming a loss of matrix integrity.

This remodeling is associated with an increased production of elastin-derived peptides (EDPs). We show that these bioactive fragments inhibit autophagy, a key cellular process for maintaining VSMC homeostasis, whose impairment contributes to the aortic manifestations of Marfan syndrome. Our results reveal decreased expression of LC3-II/LC3-I, Beclin-1, pULK1, and p62. Functional analysis (LC3-GFP transfection) demonstrates a significant reduction in LC3 puncta, reflecting reduced autophagic flux, in association with dysregulation of the mTOR, AMPKα, and PI3KC1A pathways.

In conclusion, the absence of the anti-inflammatory receptor TREM2 triggers profound alterations in the vascular extracellular matrix, thereby exacerbating the progression of Marfan syndrome. The resulting elastin peptides strongly contribute to the deregulation of autophagic flux, a process known to aggravate the aortic manifestations of this pathology.

Structure/function analysis of the glycan component of joint fluids: comparative study between osteoarthritis and rheumatoid arthritis and impact of radio-clinical phenotype

NAIM Meriem [1*], TAN Elina[2*], FRACZ Kinga[1], SAADAN Chayma Kellou[1], FLAGEOLLET Camille[1], EVEN Benjamin[1], EYMARD Florent[2] AND ALBANESE Patricia[1]

[1] Laboratoire Gly-CRRET (Glycans - Croissance, Réparation et Régénération Tissulaires), EA UPEC 4397, Université Paris Est Créteil Val-de-Marne, Créteil, France
[2] AP-HP, Université Paris Est Créteil, Service de rhumatologie, Hôpital Henri Mondor, Créteil, France
*contributed equally to the data
meriem.naim@u-pec.fr

Keywords: Glycosaminoglycans, osteoarthritis, rheumatoid arthritis, inflammation

Abstract:

Purpose: Glycosaminoglycans (GAGs) are linear sulfated polysaccharides associated with protein cores to form proteoglycans. They are present in the extracellular matrix and pericellular matrix of all tissus. They regulate pathophysiological processes by interacting with numerous regulators of tissular homeostasis that are Heparin Binding Proteins (HBP) such as growth factors, interleukins, chemokines or matrix remodeling enzymes. These interactions are dependent on the size and level of sulfation of GAG chains, controlled by over 30 biosynthesis enzymes[1]. We previously demonstrated that amount and sulfation pattern of GAG evolve in human matrix cartilage during degenerative osteoarthritis (OA) and are associated with catabolic effects on chondrocytes[2]. More recently we identified a very rare and specific sulfation signature of GAG in OA synovial fluid (SF), modulated by pro-inflammatory cytokines. To explore GAG role in joint inflammatory process, we focus further on rheumatoid arthritis (RA), a very severe inflammatory pathology in which cartilage degradation is not the first step like in OA but a consequence of matrix degradation[3]. The aims of our study are to compare the quantitative, structural and functional characteristics of SF GAG from patients with RA and OA, and to investigate correlations between these glycan signatures and patients' clinical, biological or radiographic data.

Methods: SF were collected from the BIOGO cohort (Henri Mondor Hospital, Rheumatology department), including patients with OA or RA, and their cytokine profile was characterized. GAG were purified from SF samples and quantified by DMMB. SF GAG binding affinity for different HBP was analyzed by competitive ELISA binding assays. Human synoviocytes were cultured from SF from OA patients. The impact of SF GAG (OA versus RA) was assessed on the inflammatory phenotype of synoviocytes, by ELISA assay of the secretion of various cytokines and MMPs.

Results: RA SF (n=6) showed a higher concentration of inflammatory and angiogenic mediators (VEGF: 1000±209 pg/mL, CCL5: 500±200 pg/mL, CXCL10: 570±80 pg/mL, PDGFBB: 30±18 pg/mL) as compared to OA SF (n=6) (VEGF: 234±100 pg/mL, CCL5: 140±57 pg/mL, CXCL10: 80±42 pg/mL, PDGFBB: undetected). Median GAG concentration in SF from RA (n=12) and OA (n=23) was similar (26±11 vs. 24±15 µg/mL). In RA patients, GAG level was not associated with CRP alone (p=0.11), but it was highly significantly correlated with clinical disease activity measured by DAS 28 CRP (p=0.0001 In OA patients, it was not associated with clinical (p=0.56) or radiographic (p=0.46) severity. Functionally, GAG from RA and OA SF interacted with VEGF, FGF2, PDGFBB, and PTN but with low affinity as compared to heparin and with no difference between OA and RA. Finally, GAG from RA SF induced IL-6 secretion by synoviocytes (x2) significantly higher than those induced by GAG form OA SF.

Conclusion: While there is no quantitative difference in GAG amount in SF from RA and OA patients, GAG amount from RA SF are correlated with disease activity and modulate the inflammatory phenotype of synoviocytes. These could be explained by structural specificities of GAG from RA SF, such as chain size and sulfation pattern, that are currently under investigation. Moreover, GAG from RA SF are able to bind inflammatory and angiogenic mediators present in SF. The impact of GAG on the angiogenic phenotype of endothelial cells is currently being evaluated to highlight their role in the inflammation/angiogenesis axis that characterizes this pathology.

References: [1](Ouidja *et al.*, *Essays Biochem.*, 2024)
[2](Shamdani *et al.*, *Arthritis Research and Therapy*, 2020)
[3](Alamgeer *et al.*, *Phytomedicine.*, 2020)
[4](Liu X *et al.*, *Front Immunol.*, 2022)

Ageing of the Extracellular Matrix: Impact of senescent fibroblasts secretome in Ovarian Cancer Dissemination

Luce Perié[a], Mickaëla Bignard[a], Alexandra Landras[a], , Marion Theuret[a], Mélanie Briand[b], Franck Carreiras[a], Cédric Picot*[a]

[a]*Equipe de Recherche sur Les Relations Matrice Extracellulaire-Cellules, ERRMECe EA1391, Groupe Matrice Extracellulaire et Physiopathologie (MECuP), Institut des Matériaux, I-MAT (FD4122), CY Cergy Paris Université, France*

[b] *Centre de Lutte Contre le Cancer François Baclesse, UMR 1086 « ANTICIPE » INSERM – Université de Caen Normandie, Plateforme ORGAPRED "Organoïdes Tumoraux à Visée Prédictive et de Recherche"*

* cedric.picot@cyu.fr

Keywords: ovarian cancer, senescent fibroblasts, SASP, ECM remodelling

Abstract:

The increase in global life expectancy has led to a surge in age-related diseases, including cancer, placing growing pressure on healthcare systems. Although ovarian cancer (OC) accounts for only 3–4% of female cancers, it remains the leading cause of death from gynaecologic malignancies, with age being a major risk factor. Emerging evidence highlights the critical role of the tumor microenvironment, particularly the inflammatory crosstalk between fibroblasts and macrophages, in driving OC progression. In aged tissues, this crosstalk is further shaped by the accumulation of senescent cells and their senescence-associated secretory phenotype (SASP). Macrophages secrete cytokines that not only support tumor growth and metastasis but also induce premature senescence in surrounding fibroblasts, amplifying local inflammation and ECM remodeling While senescence was initially considered as tumor-suppressive, it is now recognized as a driver of cancer progression in aged microenvironments. Senescent fibroblasts accumulate with age and promote tumor spread through the secretion of pro-inflammatory factors and an age-altered remodelling of extracellular matrix (ECM) proteins, which facilitates cancer cell invasion and sustains immune cell recruitment.

Our recent findings underscore the role of fibronectin, an ECM protein, in promoting fibroblast senescence and OC progression, echoing broader evidence that positions the ECM as a key regulator of cell communication in cancer [1–3]. Here, we aim to decipher how the senescent microenvironment influences the dissemination of ovarian cancer cells by investigating how senescent fibroblasts affect cancer cell behaviour through their secretome. In doing so, we will identify key ECM components and secreted factors that drive this process and may represent future therapeutic targets.

References:

[1] R. Bascetin, L. Blay, S. Kellouche, F. Carreiras, C.R. Picot, M. Briand, R. Agniel, O. Gallet, C. Vendrely, J. Leroy-Dudal, Fibronectin amyloid-like aggregation alters its extracellular matrix incorporation and promotes a single and sparsed cell migration, Exp Cell Res 371 (2018). https://doi.org/10.1016/j.yexcr.2018.07.047.

[2] L. Perié, C. Houël, A. Zambon, C. Guere, K. Vié, J. Leroy-Dudal, C. Vendrely, R. Agniel, F. Carreiras, C.R. Picot, Impaired incorporation of fibronectin into the extracellular matrix during aging exacerbates the senescent state of dermal cells, Exp Cell Res 442 (2024). https://doi.org/10.1016/j.yexcr.2024.114251.

[3] C. Laurent-Issartel, A. Landras, R. Agniel, F. Giffard, C. Blanc-Fournier, E. Da Silva Cruz, C. Habes, J. Leroy-Dudal, F. Carreiras, S. Kellouche, Ascites microenvironment conditions the peritoneal pre-metastatic niche to promote the implantation of ovarian tumor spheroids: Involvement of fibrinogen/fibrin and αV and α5β1 integrins, Exp Cell Res 441 (2024). https://doi.org/10.1016/j.yexcr.2024.114155.

Aortic wall alterations associated with Tenascin-X deficiency

Samuel Salque*[1], Clenildo Silva Campos[2], Laura Prigent[1], Perrine Mercier-Gouy[1], Cindy Dieryckx[1], Axel Couturier[1], Ming Lo[1], Elise Lambert[1], Luciano Figueiredo Borges[1-3], Ulrich Valcourt[1]

[1] *Laboratoire de Biologie Tissulaire et Ingénierie Thérapeutique (LBTI), UMR CNRS 5305, Université Claude Bernard Lyon 1, Institut de Biologie et Chimie des Protéines, Lyon, France.*
[2] *Laboratory of Molecular and Experimental Pathology, Federal University of São Paulo, Brazil.*
[3] *Institute for Advanced Study - Collegium de Lyon – France*

* samuel.salque@univ-lyon1.fr

Keywords: Aorta, Tenascin-X, Vascular integrity, Ehlers-Danlos Syndrome.

Abstract:

Tenascin-X (TNX) is an extracellular matrix (ECM) glycoprotein found in the connective tissues of most adult organs, including the aortic wall. TNX deficiency causes the *classical-like* subtype of Ehlers-Danlos Syndrome (*cl*EDS), a rare genetic disorder characterized by altered biomechanical properties of connective tissues. In addition to symptoms common to all EDS subtypes, *cl*EDS patients exhibit vascular fragility, including some occurrences on large vessels. Although pathological alterations in macrovessel walls have been observed in mice with vascular cell-specific TNX ablation, conflicting results have been obtained regarding the role of TNX in aortic integrity[1,2]. To clarify this, we explored the vascular phenotype in a constitutive TNX-deficient mouse model, which better reflects the *cl*EDS physiopathology.

In the descending thoracic aorta of TNX knock-out (KO) animals, the histological analysis of elastic fibers (Resorcin/Fuchsin staining) displayed no significant quantitative modification. In contrast, both total collagen density (Picrosirius staining) and collagen fibril diameter (transmission electron microscopy) were increased in the medial and adventitial layers, indicating enhanced collagen deposition within the ECM, thereby altering its normal architectural structure. Despite these structural changes, we observed no differences in the mRNA levels (RT-qPCR) of core matrix genes, such as fibrillar Collagens (types I, III, and V), Fibrillin-1 and Elastin. However, expression of Lumican and matrix metalloproteinase (Mmp)-9 were significantly upregulated, suggesting a disruption in ECM homeostasis. Additionally, isolated aortas from KO mice exposed to increasing mechanical stress (wire myography) were found to be more compliant than WT ones. This more relaxed and disorganized matrix network suggests reduced resistance to systolic pressure in physiological conditions.

We next focused on vascular smooth muscle cells (vSMCs) behaviour in the absence of TNX. Ultrastructural analysis revealed that vSMCs more frequently displayed dilated endoplasmic reticulum and collagen-rich pericellular environments compared to WT animals, suggesting a shift towards a secretory phenotype. To determine the functional consequences, we assessed the responses of isolated aortas to vasoactive drugs using *ex vivo* myography. TNX-deficient aortas exhibited a significantly (p=0.0238) enhanced vasodilatory response to acetylcholine (Emax = 60.8 ± 4.2%) compared to WT vessels (Emax = 46.2 ± 4.3%). This phenomenon is endothelium-dependent as exposition to the NO donor sodium nitroprusside gave no significant change between WT (Emax = 98.4 ± 1.5%) and KO (Emax = 101 ± 0.8%) mice. These results indicate an impaired dialogue between vSMCs and endothelial cells in the absence of TNX.

Altogether, our results highlight a crucial role of TNX in regulating ECM composition and organization, as well as vascular cell vasomotricity. To further explore the molecular and cellular mechanisms underlying these results in TNX-deficient mice, we are conducting mass spectrometry-based quantitative proteomic analyses. By characterizing both the total proteome and Matrisome, we aim to identify processes by which TNX regulates aortic integrity in physiological context. Given that TNX deficiency is implicated in aortic wall fragility and pathological ECM remodelling, this work paves the way for investigating its contribution in the development of aortopathies, such as aneurysm and atherosclerosis.

References:

[1]: G. Liang; XF. Lv; W. Huang; YJ. Jin; KA. Roquid; H. Kawase; S. Offermanns, *Arterioscler Thromb Vasc Biol.*, 2024, **44(8)**:1748-1763.
[2]: G. Liang; S. Wang; J. Shao; YJ. Jin; L. Xu; Y. Yan; S. Günther; L. Wang; S. Offermanns, *Circ Res.*, 2022, **130(11)**:1647-1659.

Modeling ovarian cancer peritoneal dissemination using 3D heterotypic tumor spheroids

Marion Theuret [a], Alexandra Landras [a], Johanne Leroy-Dudal [a], Mélanie Briand [b], Romane Florent [b], Ambroise Lambert [a], Franck Carreiras [a], Sabrina Kellouche [a*]

[a] *Equipe de Recherche sur Les Relations Matrice Extracellulaire-Cellules, ERRMECe EA1391, Groupe Matrice Extracellulaire et Physiopathologie (MECuP), Institut des Matériaux, I-MAT (FD4122), CY Cergy Paris Université, France*

[b] *Centre de Lutte Contre le Cancer François Baclesse, UMR 1086 « ANTICIPE » INSERM – Université de Caen Normandie, Plateforme ORGAPRED "Organoïdes Tumoraux à Visée Prédictive et de Recherche"*

* sabrina.kellouche@cyu.fr

Keywords: Ovarian cancer; Ascites; Extracellular matrix; Heterotypic spheroids

Abstract:

Ovarian cancer (OC) is characterized by its silent onset and its ability to spread in the abdominal cavity, leading to peritoneal metastases. This dissemination occurs through the detachment of isolated cells or multicellular aggregates, known as spheroids, from the ovarian surface epithelium. Spheroids migrate *via* ascitic fluid, accumulating pathologically in the peritoneal cavity, and implant on the mesothelial lining. The dissemination process involves dynamic interactions of spheroid cells with mesothelial cells and the mesothelial extracellular matrix (ECM), whose contributions to the implantation process remain incompletely understood. Most *in vitro* models still rely on homotypic cancer cell aggregates while spheroids isolated from ascites are known to contain both cancer and stromal cells (e.g., fibroblasts, macrophages, platelets). Hence, it is challenging to encompass the *in vivo* complexity of tumor microenvironment.

We developed an ascitic 3D heterotypic spheroid model combining ovarian cancer cells and human mesothelial cells, which mimic the structural and functional features of patient-derived spheroids. This strategy enables us to model the early steps of peritoneal dissemination in OC using 3D heterotypic spheroids and to investigate how mesothelial cells and ECM components modulate spheroid behavior and implantation.

Preliminary results show that mesothelial cell type and their relative amount compared to tumor cells influence spheroid architecture. The addition of mesothelial cells tunes spheroid compaction which might also shift adhesive and invasive properties (Figure 1). Heterotypic spheroids provide a relevant 3D model to study the cellular and matrix interactions involved in OC peritoneal dissemination, offering new insights into the implantation process and potential therapeutic targets.

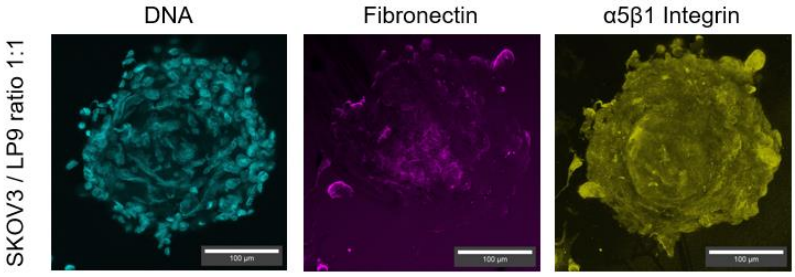


Figure 1: Heterotypic ovarian cancer spheroids (SKOV3 / LP9) were seeded on fibronectin-coated surfaces and incubated in ascites. After 4h of incubation, immunofluorescent staining of Fibronectin (magenta), α5β1 Integrin (yellow) and DNA labeling (blue) were done. Scale bar: 100 µm.

SESSION 2

"Assemblage & dynamique de l’ECM"

Conférences invitées

From a collagenocentric to a more integrated view of matrix biology in development, repair and disease

Florence Ruggiero

Institut de Génomique Fonctionnelle de Lyon (IGFL)
Ecole Normale Supérieure de Lyon.

* florence.ruggiero@ens-lyon.fr

Keywords: extracellular matrix topology, functional genomics, live imaging, zebrafish

Abstract:

For decades, the ECM was cast as inert scaffolding. Today, thanks to relentless work by the matrix community and bold new entrants, that view is obsolete. The extracellular matrix (ECM) is a dynamic command center, directing stem cell fate, transmitting mechanical forces, and shaping inflammation, metabolism, and regeneration. In my lab, we pursue a core question: how do cells build and remodel 3D tissue-specific ECMs in real time, and how do they respond in this ongoing molecular conversation? Our starting point was the collagen superfamily, where we revealed structural and signaling diversity and the devastating impact of collagenopathies. This work reinforced a deeper truth: the ECM is not a pile of proteins but an integrated network demanding systems-level investigation with the most advanced tools available. Enter the zebrafish, our living, regenerating window into ECM biology and pathology. With genome editing, functional genomics, live imaging, and behavioral assays, we track ECM dynamics from single genes to whole organism, from embryos to adults. Our current focus includes scar-free skin repair, neuromuscular development, regeneration, and diseases. The picture is clear: cells build complex, 4D tissue-specific ECM scaffolds for embryonic development, and the ECM is a versatile, constantly remodeled network that sustains life. Yet the journey is far from over, the ECM still guards its secrets, and matrix scientists are determined to uncover them. The opportunity is great: to connect fundamental ECM biology with new therapeutic strategies for human disease.

Peptidic models of the collagen tripple helix

Abhishek Jalan[a], Audrey Ziverec[b], Frédéric Mallein-Gerin[b], Jean-Daniel Malcor*[b]

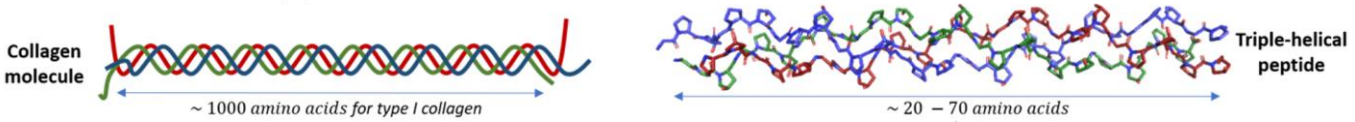
[a] *Department of Biomaterials, University of Bayreuth, Germany*
[b] *Laboratory of Tissue Biology and Therapeutic Engineering, CNRS UMR 5305 University of Lyon, France*

* jean-daniel.malcor@ibcp.fr

Keywords: collagen, triple helix, cell-matrix interactions

Abstract:
On a molecular level, collagens adopt a triple helix conformation. This rod-like motif is composed of three polypeptides (also called α-chains) that each contains a polymeric amino acid repeat where glycine occupies every third position. This unique sequence enables the stacking of collagen molecules into supramolecular structures, such as fibrils or mesh-like networks, that shape the mechanical, architectural and biophysical properties of the extra-cellular matrix (ECM). The triple-helical domains of collagen are also crucial to interactions with transmembrane receptors, enzymes, chaperones and secreted ECM components, to regulate complex cellular processes such as cellular attachment to the ECM, proliferation, cell migration and cell differentiation. Thus, understanding the triple helix structure and its interactions with cells is key to elucidate how collagen regulates tissue homeostasis.

However, collagens are large and highly complex proteins that often form aggregates upon extraction from native tissues, making experimentation with whole molecules unpractical. Instead, synthetic peptides with a sequence composed of $(GXY)_n$ triplets that spontaneoulsy bundle into a triple-helix are chemically defined and make convenient tools to mimic collagen.[1] These THPs have been used to map the binding sites of homotrimeric collagens Col2 and Col3, leading to the identification of recognition motifs for collagen-binding receptors, including the collagen-binding integrins (α1β1, α2β1, α10β1 and α11β1), Discoidin Domain Receptor (DDR) 1 and 2, Leukocyte-associated immunoglobulin-like receptor 1 (LAIR-1), Glycoprotein VI, von Willebrand Factor (vWF) and the SPARC protein.



THPs that integrate such motifs have been employed to investigate the specific impact of these proteins in cell behaviour, and highlight their role in disease. As a result, THPs have enabled tissue engineers to address collagen-binding receptors in cell-laden biomaterials and optimise outcomes for subsequent regenerative medicine applications.[2] We have notably grafted integrin- and DDR-binding THPs to biologically inert biomaterials, in order to significantly improve their interactions with endothelial cells, mesenchymal stem cells or chondrocytes.[3, 4] Furthermore, THPs have been pivotal models to characterize the triple helix structure. We have thus demonstrated that inter-strand salt bridges contribute to the correct folding of the collagen triple helix, and then keeps it folded despite interruptions within the triple helical domains.[5]

References:

[1] R.W. Farndale, Collagen-binding proteins: insights from the Collagen Toolkits, Essays in biochemistry 63(3) (2019) 337-348.
[2] J.-D. Malcor, F. Mallein-Gerin, Biomaterial functionalization with triple-helical peptides for tissue engineering, Acta Biomaterialia 148 (2022) 1-21.
[3] J.-D. Malcor, E.J. Hunter, N. Davidenko, D.V. Bax, R. Cameron, S. Best, S. Sinha, R.W. Farndale, Collagen scaffolds functionalized with triple-helical peptides support 3D HUVEC culture, Regenerative Biomaterials 7(5) (2020) 471-482.
[4] M. Colzani, J.D. Malcor, E.J. Hunter, S. Bayraktar, M. Polkinghorne, T. Krieg, R. Cameron, S. Best, R.W. Farndale, S. Sinha, Modulating hESC-derived cardiomyocyte and endothelial cell function with triple-helical peptides for heart tissue engineering, Biomaterials 269 (2020) 120612.
[5] J.-D. Malcor, N. Ferruz, S. Romero-Romero, S. Dhingra, V. Sagar, A.A. Jalan, Deciphering the folding code of collagens, Nature Communications 16(1) (2025) 2702.

Communications orales

Refined cellular models for extracellular matrix-related neuromuscular disorders

Dimitrios Kourtzas[a], Zoheir Guesmia[a], Jessica Ohana[a], Satish Moparthi[a], Bruno Cadot[a], Anne Bigot[a], Stéphane Vassilopoulos[a], Valérie Allamand*[a]

[a] *Sorbonne Université, Inserm, Institut de Myologie, Centre de Recherche en Myologie, Paris, France*

* valerie.allamand@inserm.fr

Keywords: Neuromuscular disorders, Collagens, Fibrosis, Myomatrix

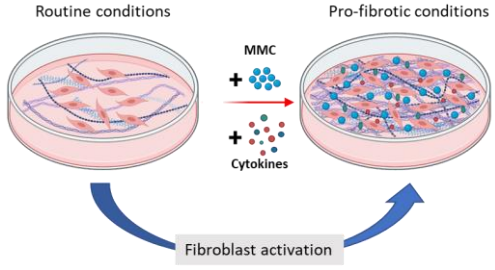
Abstract:

Extracellular matrices (ECM) hold cells together in tissues and constitute a unique and specific microenvironment for cells. In skeletal muscle, the ECM surrounding myofibers, referred to as myomatrix [1], is composed of different layers: a thin basement membrane (BM) that is in direct contact and interacts with the sarcolemma, and a collagen-rich interstitial stroma. Collagen type VI (COLVI) beaded microfilaments are located at the interface between these layers, ensuring the architectural integrity of the myomatrix, anchoring cells and mediating, directly or indirectly, signalling pathways through transmembrane receptors [2]. COLVI deficiency, due to mutations in the *COL6A1-3* genes, leads to rare neuromuscular disorders, collectively termed COL6-related dystrophies (COL6-RD) for which, to date, there is still no curative treatment [3].

A significant histological signature of COL6-RD is skeletal muscle fibrosis – *i.e.* exacerbated accumulation of ECM components in the interstitium, such as collagens and fibronectin. COLVI itself is a biomarker of fibrosis but it is also recognized as an important driver of fibrosis, notably through cleavage of the α3(VI) chain C-terminal domain [4].

Since 2009, a relevant model to mimic fibrogenesis *in vitro* has emerged: the so-called "scar-in-a-jar-system", which relies on the biophysical effect of macromolecular crowding to recapitulate the micro-environmental constraints applied to cells in tissues, and on the exogenous addition of cytokines to accelerate and enhance ECM deposition [5]. We sought to adapt and optimize this system for COL6-RD research, by testing different macromolecular crowding agents and cytokines. Dermal fibroblasts – commonly used for COL6-RD diagnostic procedures – and fibro-adipogenic precursors (FAPs) – the main ECM-producing cells in skeletal muscle [6] – cultured in these pseudo-3D conditions display enhanced expression of collagen type I, a prototypic fibrosis marker, and other ECM molecules, including COLVI. Using complementary readouts we analyzed the deposited ECM, and the impact of these culture conditions on cellular homeostasis.

In conclusion, these refined culture conditions provide novel pro-fibrotic cellular models for neuromuscular disorders. These *in vitro* models are valuable tools for detailed analysis of the consequences of *COL6A1-3* genetic variants on ECM composition, organization and remodelling. Further, these models will be useful for the development and validation of therapeutic strategies for COL6-RD.



Induction of pro-fibrotic conditions.
The addition of macromolecular crowder(s) (MMC) and cytokines leads to the activation of fibroblasts into myofibroblasts and the induction of a pro-fibrotic environment.

References:

[1]: A. Rutkowski, C. Bönnemann, S. Brown S, et al., *Neuromuscular Disorders,* 2013, **23(2)**,188–191.
[2]: SR. Lamandé and JF. Bateman, *Matrix Biology,* 2018, **71-72**, 348-367.
[3]: P. Mohassel, AR. Foley, CG. Bönnemann, *Matrix Biology,* 2018, **71-72**, 188-204.
[4]: L. Williams, T. Layton, N. Yan, et al., *The FEBS Journal,* 2021, **289(13)**, 3603–3629.
[5]: CZC. Chen, YX. Peng, ZB. Wang, et al., *British Journal of Pharmacology,* 2009, **158(5)**, 1196–1209.
[6]: Y. Zou, R. Zhang, P. Sabatelli, et al., *J Neuropathol Exp Neurol*, 2008, **67(2)**, 144-164.

Characterization of the metabolic profile of skin repair in relation to extracellular matrix expression

Alexandra Pavilla[1*], Didier Pin[2], Bertrand Perrin[3] and Patricia Rousselle[1]

[1] *Tissue Biology and Therapeutic Engineering Unit, Institute of Protein Biology and Chemistry, UMR 5305 – CNRS, University of Lyon, Lyon Cedex 07, France*
[2] *VetAgro Sup, Dermatology Unit, 69280, Marcy l'Etoile, France*
[3] *Cohesives, R&D laboratory, 21000 Dijon, France*

* alexandra.pavilla@ibcp.fr

Keywords: ECM ; wound repair ; metabolism

Abstract:
Throughout life, multicellular organisms, from invertebrates to mammals, are exposed to mechanical injury and infection. In mammals, the injury-induced response is a reparative process that results in a permanent replacement of the damaged tissue with a collagenous connective tissue called a scar. Most wounds heal through a coordinated temporal sequence of coagulation, inflammation and clearance of pathogens and necrotic debris, followed by local proliferation of the cells required to repair the wound through synthesis and remodeling of the extracellular matrix (ECM). Although it is evident that the metabolic requirements of cells in the tissue change during healing, little is known about the cell-intrinsic and -extrinsic metabolic adaptations necessary for wound healing and tissue repair. All cells involved require energy to grow and proliferate, maintain their structures and respond to environmental changes. They respond to highly regulated bioenergetic processes and produce energy and biosynthetic precursors to maintain cellular homeostasis and function. Metabolism is crucial for the control of cellular functions, in particular through mitochondrial and cellular metabolic processes as well as substrate oxidation and ATP production. In our work, we developed a strategy to assess tissue metabolism during skin healing in a deep porcine wound model under two different wound treatment conditions. In parallel, we evaluated the expression of biomarkers of inflammatory cells and events involved in tissue repair, such as angiogenesis, re-epithelialisation, granulation tissue formation and ECM remodeling. By measuring cellular oxygen consumption rates (OCR) in real time on freshly harvested tissue sections, we were able to report on the monitoring of mitochondrial respiration at different stages of the healing process. Our work shows that the mitochondrial respiratory profile of the tissue not only varies over time but also depends on the treatment of the wound. We also show that this profile correlates with cellular responses specific to each stage of the tissue repair process and ECM production. We hypothesize that characterizing the metabolic changes that occur in skin wounds will contribute to the development of efficient therapeutic options.

Ascorbic acid and hypoxia promote collagen deposition through distinct mechanisms

Méline Ricol [a], Sandrine Vadon-Le Goff [a], Jean-Baptiste Vincourt [b], Catherine Moali *[a], Violaine Sée *[a]

[a] *Tissue Biology and Therapeutic Engineering Laboratory, UMR5305, 69367 Lyon, France*
[b] *University of Lorraine, CNRS, INSERM UMS2008, IBSLor, Proteomics core facility, Vandoeuvre-lès-Nancy, France*

* catherine.moali@cnrs.fr, violaine.see@univ-lyon1.fr

Keywords: collagen I, hydroxyproline, BMP1, HIF

Abstract:
The biosynthesis of fibrillar collagens is tightly regulated and can be activated through various stimuli. Among those triggers, ascorbic acid (AA) is a commonly used agent to boost collagen biosynthesis in cell culture, as a cofactor of the collagen prolyl hydroxylases. Another trigger playing a major role in the context of fibrotic disorders such as systemic sclerosis, is hypoxia. Here, we wondered how AA and hypoxia, both part of the cellular microenvironment, work independently or in synergy to control collagen production. We compared the mechanisms induced by these two stimuli using human primary dermal fibroblasts as a model system.

We initially measured collagen I deposition, and observed a higher staining for collagen I in 2D cell cultures supplemented with AA compared to cultures in hypoxia (1% O_2). Combining both stimuli resulted in additive effects but neither AA nor hypoxia significantly influenced procollagen I transcription. In contrast, both increased the hydroxylation of procollagens by prolyl hydroxylases, although through distinct mechanisms, resulting in increased levels of secreted procollagens as detected by proteomic analysis, Western Blot and hydroxyproline quantification.

Tansmission electron microscopy also revealed different properties of assembled collagen fibres and associated ECM with AA or hypoxia and this prompted us to analyse the proteolytic maturation of procollagens in the two conditions. Surprisingly, the removal of collagen I C-propeptide, which depends on the BMP1/tolloid-like proteinases (BTPs) and is a critical step for collagen integration into the matrix, differed between the two stimuli. In hypoxic conditions, BTP inhibition completely abolished the increase in collagen deposition whereas, in cells treated with AA, BMP1 inhibition did not prevent collagen integration into the matrix. Furthermore, our secretome analysis revealed that several partners susceptible to influence collagen biosynthesis are differentially modified between AA and hypoxia, suggesting additional regulatory mechanisms which are currently under investigation.

Altogether, these results show that distinct mechanisms are involved in collagen deposition by fibroblasts depending on the microenvironmental factors. Additionally, this raises the possibility that other proteases, yet to be identified, could be responsible for the cleavage of the collagen C-propeptide, challenging the long-standing belief in the requirement for BTPs. Finally, our results open interesting perspectives for the selective inhibition of BTPs in fibrotic disorders that involve hypoxia as a key driving factor.

The respective positional substrate specificities of procollagen proly-4-hydroxylase isoenzymes control tissue-specific procollagen hydroxylation and further maturation

Déborah HELLE [a], Alison WURTZ [a, c], Hanane ABOUELFARAH [a, c], Anne ROBERT [a], Dafné WILHELM [a, c], Nick RAMALANJAONA [a], Catherine BUI [a], Sandrine GULBERTI [a], Jean-Baptiste VINCOURT [a, b *].

[a] *Université de Lorraine, CNRS, IMoPA, UMR 7365, Nancy, France*

[b] *Proteomics core facility of Pôle Biologie-Médecine-Santé from the University of Lorraine, Nancy, France*

[c] *These authors have left the laboratory at the time of submission*

* jean-baptiste.vincourt@univ-lorraine.fr

Keywords: post-translational modification; triple helix; P4HA, PLOD

Abstract:

Type I collagen, the most abundant protein of vertebrate organisms, is polymerized into a macroscopic-scale fibrillar network, the detailed arrangement of which is controlled by numerous post-translational modifications (PTMs) to determine the biomechanics of connective tissues. Its most frequent post-translational modification, prolyl-4-hydroxylation (P4H), catalysed by prolyl-4-hydroxylases (P4HAs) and occurring at more than 100 residues per procollagen alpha chain, is crucial to thermodynamically stabilizing its triple helical building block. Therefore, it was considered a ubiquitous process, until we reported distinct positional profiles of collagen P4H between bone, skin and tendon tissues, suggesting that collagen P4H profiles contribute tissue-specific biomechanics [1].

Here, we report the respective contributions of P4HA1 and P4HA2 to procollagen PTM. Using a dedicated cellular model of collagen assembly together with adenoviral transduction of CRISPR/Cas9 constructs and label-free relative quantitative proteomics, we find that both enzymes share most XPG sites of type I collagen as potential substrates. Indeed, each single mutant achieves overall P4H levels comparable to controls, while double mutants exhibit drastically reduced levels. Nevertheless, each isoform exhibits 5-10 specific substrate residue positions within the whole procollagen alpha chains. Remarkably, the P4HA1 specific substrates must play a particular role in triple helix stabilization, as their lack of hydroxylation results in drastically reduced collagen assembly. We find that the substrate specificity of P4Has is defined partly, but not mostly, by the nature of the surrounding X residue within XPG collagen typical triplets. The substrate specificities of P4HA1 and P4HA2 correlate to the specific 4-hydroxylation profiles observed in the skin and the tendon, respectively, highlighting these enzymes as major contributors to defining tissue-specific P4H profiles. Furthermore, based on genetic and metabolic engineering, we demonstrate that P4HA activity controls other collagen PTMs (prolyl-3-hydroxylation and lysyl-glycosylation) contributing to defining tissue-specific collagen maturation and biomechanics. Specifically, P4HA activity favours prolyl-3-hydroxylation but inhibits lysyl glycosylation. These findings highlight the strategic molecular function of P4HAs within the collagen maturation machinery, for steering tissue-specific collagen assembly.

Recently, others published studies of the substrate repertoire of P4HAs, but came to distinct conclusions in detail [2, 3]. We discuss the molecular mechanisms dictating the positional substrate specificity of P4HAs, their possible consequences over the dynamics of the collagen triple helix formation, and how all this, collectively, controls further modifications of collagen, and thereby, the biomechanics of connective tissues.

References:

[1] D. Wilhelm, A. Wurtz, H. Abouelfarah, G. Sanchez, C. Bui, J.B. Vincourt, *Matrix Biol* 2023, **119**, 141-153.
[2] A.M. Salo, P. Rappu, M.K. Koski, E. Karjalainen, V. Izzi, K. Drushinin, I. Miinalainen, J. Kapyla, J. Heino, J. Myllyharju, *Matrix Biol* 2023,
[3] V. Sarohi, *Front Cell Dev Biol* 2025, **13**, 1527839.

Posters

Structural effects of pulsed electric fields on collagen secreted by dermal fibroblasts

Emma Barrère [a], Nicolas Mattei [a], Ophelie Cordier [a], Marie-Pierre Rols [a], Muriel Golzio [a], Jelena Kolosnjaj-Tabi *[a]

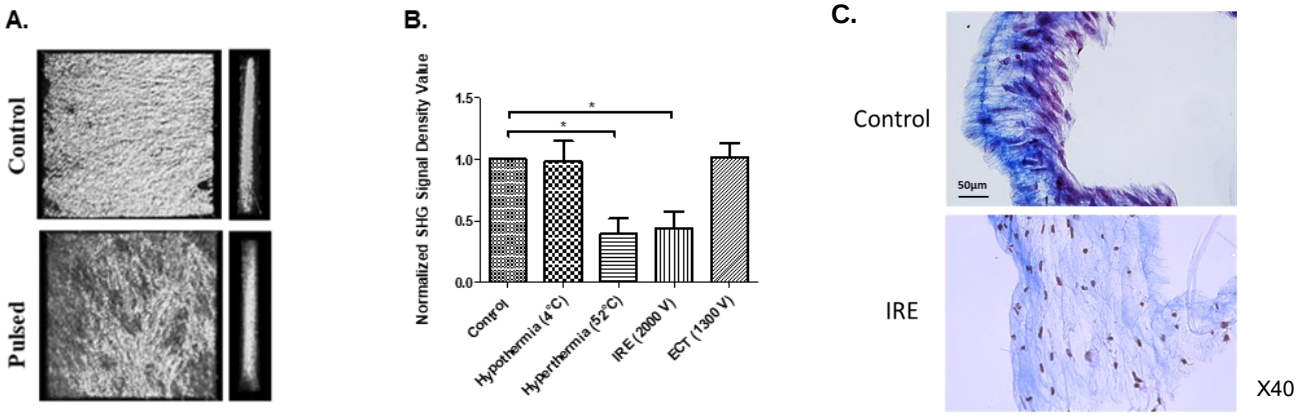
[a] *Institute of Pharmacology and Structural Biology, 205 route de Narbonne, 31077 Toulouse, FRANCE (CNRS UMR 5089)*

* Jelena.Kolosnjaj-Tabi@ipbs.fr

Keywords: pulsed electric fields; collagen; fibroblasts; cancer

Abstract:

Pulsed electric fields can permeabilize the cell membrane. While such membrane effects, also known as “electroporation”, have been studied and exploited in therapy over the last decades, the direct effects of electric pulses on the extracellular matrix (ECM), namely the collagen are sparsely documented.
Nevertheless, increased collagen production can be involved in tumor development, dissemination, and metastasis progression [1]. In addition, collagen can act as a barrier, which prevents the penetration of therapeutics and immune cells into the tumor. Finding a strategy that would make the collagen more permeable, might benefit cancer patients. Here we thus evaluated if irreversible electroporation (IRE)-based protocols can directly alter the structure of the ECM.
Using engineered dermal sheets as a 3D biological model, characterized by a self-secreted collagen matrix and serving previously to evaluate the indirect effects of reversible electroporation [2], we show that the application of IRE pulses (80 x 100 µs, 2000 V/cm, 1 Hz) yields to a loss of collagen signal density, detected by second harmonic generation (SHG) microscopy after treatments (<3h) (Fig A and B). These results are similar to hyperthermia exposure (1 h, 52°C) used as a positive control, known for its impact on collagen fibers structure [3], whereas hypothermia (1 h, 4°C) was used as a negative control. The temperature during IRE protocol remained below 32°C suggesting a direct destructurating effect of IRE treatments on the collagen matrix. This puling condition was compared to reversible electroporation electrochemotherapy (electrochemotherapy-like pulses: 8 x 100 µs, 1300 V/cm, 1 Hz), that didn’t show any significative direct impact on collagen density (Fig B).
This work highlights the potential of pulsed electric fields to directly alter the collagen structure, indicating that applied IRE treatments could modulate collagen matrix organization. This finding appears promising as it indicates that IRE pulses could potentially be used to prime the extracellular matrix, being a potential solution to disorganize the collagen and allow a better penetration of immune cells or therapeutic agents into fibrotic tumors.



Impact of the treatments on dermal-sheets-containing collagen, determined by SHG microscopy. A: SHG microscopy images of control and IRE condition. Collagen appears in light grey. B: Quantification of the SGH signal density, normalized to the control. Results show the mean of three independent experiments for thermal treatments and pulsed treatments. Each independent experiment contains three biological replicates. C: Histological sections after Masson Trichrome staining (mag x40).

References:

[1] T. R. Cox, The matrix in cancer. *Nature Reviews Cancer,* 2021, **21**, 217–238
[2] S. Gouarderes et al. *Journal of Investigative Dermatology*, 2022, **142**, 1326-1337
[3] J. Kolosnjaj-Tabi et al. *Pharmacol Res.*, 2017, **126**, 123-137

Extracellular matrix complexity in reconstructed human adipose tissue

Sara Gouardères[a], Yannick Jeanson[a], Isabelle Fourquaux[b], Laure Gibot[c], Frédéric Deschaseaux[a], Jean-Christophe Pages[a]

[a] *Institut RESTORE, Université de Toulouse, CNRS U-5070, Toulouse, France*
[b] *Centre de Microscopie Électronique Appliquée à la Biologie, CMEAB, Toulouse, France*
[c] *Softmat, Université de Toulouse, CNRS U-5623, Toulouse, France*

* sara.gouarderes@inserm.fr

Keywords: ECM, Mesenchymal stem cells, self-assembly

Abstract:

Mesenchymal stem cells (MSC) have been described as a heterogeneous population of postnatal, self-renewing, and multipotent progenitor cells able to differentiate towards various lineages. Adipose tissue is an accessible and abundant source of mesenchymal stem cells that can be used for soft-tissue reconstruction. Multiple approaches have been developed to generate functional adipose tissue models, mostly lacking of relevant vascular and matrix compartment, which are nevertheless essential to support and maintain tissue function and metabolic homeostasis [1]. In this study, adipose-derived stem cells were isolated from human white adipose tissue then culture during 4 weeks in a medium supplemented with ascorbic acid in order to stimulated the stromal cells to produce and organize their own “biomaterial” in the form of extracellular. After one week of culture, half of the samples were change to an adipogenic medium allowing cell to differentiate into mature beige adipocytes to obtain non-differentiated prevascularized stromal tissue (hrST) and reconstructed adipose tissue (hrAT). Changings occurring in cell metabolisms, and extracellular matrix composition were analyzed by western blotting, proteomics and oximetric measurements. Results obtained shown that ASCs derived from WAT were successfully differentiated into mature adipocytes with beige phenotype and function, including high uncoupling protein 1 (*UCP1*) protein expression and increased metabolic activity (basal oxygen consumption, proton leak, and maximum respiration). RNA and protein expression analysis confirm the beige phenotype of the reconstructed tissues and demonstrate adipokine secretion, particularly leptin, adiponectin and mimecan. Proteomics analysis further revealed the extracellular matrix as a key player in the tissue’s molecular signature after differentiation. We identified more than 300 proteins from core and matrix-related proteins in both hrST and hrAT. Among them, 19 collagens subunit, component of the five big families of collagens. Most abundant collagens identified was type I and VI, similarly to native adipose tissue ECM [2]. Upon tissue differentiation, 78 and 65 matrisome genes were respectively over and down expressed. Other ECM non-collagenous proteins identified in the adipose tissue substitute included 16 proteoglycan and over 100 glycoproteins implicated in cell-matrix and matrix-basement membrane interactions; particularly fibronectin and elastic fibers. A transition from a fibrillar to a laminar matrix constitution were observed after tissue differentiation, supported by a significant shift occurring in integrins types and overexpression of basal membranes proteins. Cross-linking enzymes LOXL2, LOXL3 and TGM2 were also found to be overexpressed in hrAT. The cell-sheet models constitute then a valuable platform to study extracellular matrix remodeling in an exclusively autologous matrix and vascular microenvironment.

References:

[1] I. V. de Sousa Neto, J. L. Q. Durigan, A. S. R. da Silva, et R. de Cássia Marqueti, « Adipose Tissue Extracellular Matrix Remodeling in Response to Dietary Patterns and Exercise: Molecular Landscape, Mechanistic Insights, and Therapeutic Approaches », *Biology (Basel)*, 2022, **vol. 11**, n° 5, p. 765.
[2] S. Y. Chun ***et al.***, « Preparation and Characterization of Human Adipose Tissue-Derived Extracellular Matrix, Growth Factors, and Stem Cells: A Concise Review », *Tissue Eng Regen Med*, 2019, **vol. 16**, n° 4, p. 385- 393.

Insight into Procollagen I C-terminal maturation through structural and biochemical studies

Oskar Lipiński [a], Cindy Dieryckx [a], Natacha Mariano [a], Loïc Carrique [b], Sandrine Vadon-Le Goff [a], Catherine Moali [a*].

[a] *Universite Claude Bernard Lyon 1, CNRS, Tissue Biology and Therapeutic Engineering Laboratory (LBTI), UMR 5305, Lyon, France*
[b] *Division of Structural Biology, University of Oxford, Oxford, UK*

* catherine.moali@cnrs.fr

Keywords: collagen, proteolysis, fibrosis

Abstract:

The proteolytic conversion of soluble procollagens into mature collagen protomers is a critical step to decrease their solubility and trigger collagen fibril formation, a key step in fibrillar collagen biosynthesis. It relies on the controlled cleavage of their C-terminal propeptides by BMP-1 and other tolloid-like metalloproteases. PCPE-1 (procollagen C-proteinase enhancer 1) enhances this process by binding conserved lysine residues on the C-propeptide, near the cleavage site, promoting asymmetric cleavage of collagen chains.

To investigate these mechanisms, we designed, produced, and purified recombinant mini-procollagens I–III, truncated versions of procollagens that retain key properties of their full-length counterparts [1]. Mini-procollagen I (Mini-I) is a heterotrimeric construct formed of two α1 and one α2 truncated chains, mimicking procollagen I. Biochemical assays showed that Mini-I exhibits preferential cleavage of its α2 chain by BMP-1 and that this bias is slightly corrected in the presence of PCPE-1. In addition to the canonical cleavage site of procollagen I, mass spectrometry also revealed another cleavage site in Mini-I's α1 chains, previously unassigned to BMP-1 activity.

Cryo-electron microscopy studies led to the first high resolution structure of a hetero-trimeric C-propeptide. The ongoing experiments aim to provide direct experimental structural information into the C-propeptide - PCPE-1 interaction, complementing AI modelling and mass spectrometry-based approaches.

Future efforts will focus on assembling the full Mini-I/BMP-1/PCPE-1 complex for high-resolution cryo-EM analysis, leading to a better understanding of collagen maturation and its therapeutic implications in fibrosis and connective tissue disorders.

References:

[1]: N. Mariano, C. Dieryckx, A. Tessier, J.-B. Vincourt, S. Vadon-Le Goff, C. Moali, *BioRxiv*, 2022, 11.10.516045

SESSION 3

"ECM & technologies pour la santé"

Conférences invitées

Cell-assembled extracellular matrix (cam) for the production of human textiles

Nicolas L'Heureux [a]

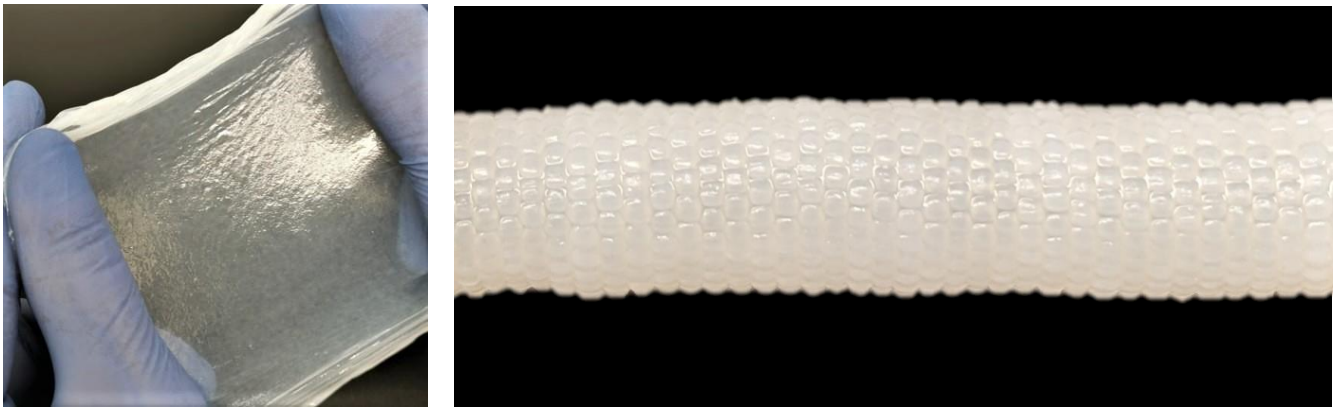
[a] *BioTis – Laboratory for the Bioengineering of Tissues, UMR1026 INSERM / Université de Bordeaux*

Nicolas.L'Heureux@Inserm.fr

Keywords: vascular, graft, cell culture, textile

Abstract:

Unfortunately, even the most inert synthetic polymers are recognized by the innate immune system as a foreign body to be destroyed. In many applications, this foreign body reaction will cause complications like obstructive fibrosis and thrombosis as a result of the chronic inflammation it generates. In addition, synthetic materials are prone to infection and have non-physiologically high stiffness. An alternative approach is to use biological materials to provide a scaffold that the body can recognize and work with. However, this means that the body's adaptive and innate immune system will also recognize and destroy, respectively, xenogeneic proteins and extracellular matrix (ECM) proteins that have been denatured (i.e., damaged). This is why animal-derived implants are treated with powerful crosslinking agents, making them unrecognizable to the cells but transforming them in a sort of foreign material. This also explains why extracellular matrix proteins that have been chemically solubilized and re-assembled are rapidly degraded after implantation. Another alternative is to have human cells assemble an ECM *in vitro* that can be used as a strong, unprocessed, completely biological, human scaffold for tissue engineering and/or surgical repair. This talk will present how Cell-Assembled extracellular Matrix (CAM) can be produced as a sheet, as a thread, and as particles to provide a new toolbox to address various regenerative medicine challenges. Past, present, and future applications will be discussed with a special emphasis on the development of human textiles [1] to produce small diameter vascular grafts [2].



Left: A CAM sheet. Right: A small-diameter vascular graft made by weaving threads of CAM [2]

References:

[1] L. Magnan; G. Labrunie; M. Fenelon; N. Dusserre; M.P. Foulc; M. Lafourcade; I. Svahn; E. Gontier; V.J. Velez; T.N. McAllister; and N. L'Heureux, *Biomateriala*, 2020, **105**, 111-120.

[2] G. Roudier; M. Hourques; N. Da Silva; M. Gluais; E. Binyet; J.-M. Olive; and N. L'Heureux, *Biofabrication*, 2024, **16**, 015015.

Biophysical investigation of skin structure

Gwendal Josse

Laboratoires Pierre Fabre

gwendal.josse@pierre-fabre.com

Keywords: skin, imaging, spectroscopy, mechanical properties

Abstract:
In this presentation, we aim at giving an overall review of the main biophysical methods appropriate for analysing skin structure and specifically skin matrix organization. The talk will emphasize on *in-vivo* measurement and imaging methods.

Several microscopy techniques are classically used on skin samples. Electronic microscopy enables the imaging of collagen fibres. Skin immuno-staining on histological slices is a classical approach for qualitatively visualizing skin matrix density. By multi-photon microscopy, elastin and collagen networks are directly imaged without any staining, and their 3D organization can be visualized. The modification of matrix organisation with age has been analysed by multi-photon microscopy [1].
The skin biochemical properties can be investigated by optical spectroscopic methods: Fourier Transform Infra-Red Spectroscopy and Raman Spectroscopy [2-3].
Several imaging techniques have been optimized for *in-vivo* skin measurement. High frequency ultrasound imaging (ultrasound at 20MHz and higher frequencies) is a quick and simple imaging technique. The multiple interaction of the ultrasound wave with the skin fibres lead to a speckle structure in the images that depends on the matrix organization. This approach has also been used to investigate the modification of skin matrix with aging [4].
Several optical methods have been also used: Reflectance Confocal Microscopy and Optical Coherence Tomography give direct access to skin image at different depths. Although these techniques use infra-red light, they are hampered by light absorption in the tissue. Other optical methods based on light diffusion in the skin can depict the strong anisotropic organisation of the skin matrix.
Skin mechanical measurement is a functional property that strongly depends on the skin matrix structure. Several devices exist for measuring *in-vivo* the skin mechanical properties, such as the Cutometer. Several research investigated the modification of skin mechanical properties with age [5]. The unusual mechanical properties of skin matrix have been studied by elastography, a technique that combines ultrasound imaging and skin mechanical testing [6].
Diverse physical properties, such as thermal analysis, have also been shown to be informative on the skin structure [3].

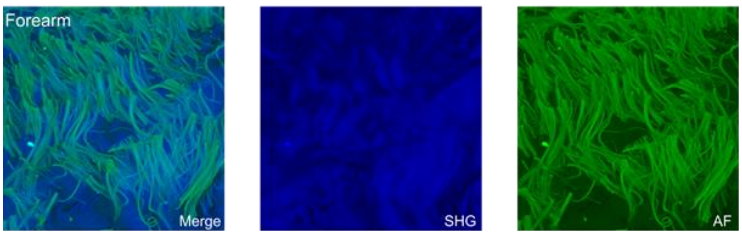


Figure : Maximum intensity projection images of multiphoton skin image (SHG : second-harmonic generation - AF : auto-fluorescence)

References:

[1] Le Digabel & al *Dermal fiber structures and photoaging*,J Biomed Opt. 2018 Sep;23(9):1-12.
[2] Villaret A,& al *Raman characterization of human skin aging.*Skin Res Technol. 2019 May;25(3):270-276
[3] Tang R, & al *Identification of ageing biomarkers in human dermis biopsies by thermal analysis (DSC) combined with Fourier transform infrared spectroscopy (FTIR/ATR).* Skin Res Technol. 2017 Nov;23(4):573-580.
[4] Sciolla B al *Joint segmentation and characterization of the dermis in 50 MHz ultrasound 2D and 3D images of the skin*. Comput Biol Med. 2018 Dec 1;103:277-286.
[5] Wan-Hsuan Lu & al *Associations of Skin Biomechanical Properties With Biological Aging Clocks and Longitudinal Changes in Intrinsic Capacity in Adults Aged 20-93:* Aging Cell. 2025 Aug 10:e70190.
[6] Mofid Y & al *Mechanical skin thinning-to-thickening transition observed in vivo through 2D high frequency elastography*. J Biomech. 2010 Nov 16;43(15):2954-62

Molecular modelling, virtual reality and AI: real assets for observing, understanding and deciphering the multi-scale interactions of the ECM

Joel Sanchez, Manon Ragouillaux, Alexandra Fontaine, Sébastien Almagro, Nicolas Belloy, Jean-Marc Crowet, Manuel Dauchez, Jessica Jonquet, Anne-Elisabeth Molza, Hua Wong, Laurent Debelle, Stéphanie Baud *

Université de Reims Champagne-Ardenne, CNRS, MEDyC UMR 7369, 51097 Reims

* stephanie.baud@univ-reims.fr

Keywords: Multi-scale modelling, Simulation, Scientific visualization, Machine learning

Abstract:

The extracellular matrix (ECM) is a complex three-dimensional network of macromolecules that provides architectural support for cells and tissue cohesion. This dynamic structure regulates numerous biological functions such as cell adhesion, migration, proliferation, differentiation, and survival. Alterations in the composition, abundance, structure, or mechanics of the ECM have been associated with diseases and disorders affecting physiological systems, including fibrosis, cancer, cardiovascular, and metabolic diseases. Thus, deciphering the protein composition of the ECM and its evolution in pathophysiological contexts appears to be a first step toward understanding the rôle of the ECM in health and disease, and toward developing therapeutic strategies to correct ECM alterations responsible for diseases.

Along the same lines, the MIME team (MEDyC research unit) has focused its research on answering the general scientific question: "How does a cell interact at the interface of its matrix microenvironment?" Among the challenges the team faces in answering this question is the fact that ECM proteins are characterized by unique biochemical properties that have hindered their study: they are bulky, heavily and particularly post-translationally modified, and highly insoluble. Thus, the adopted strategy is to visualize, model, and understand all associated levels of complexity: atomic and molecular, mesoscopic, and tissue. To achieve this, theoretical and experimental biophysical approaches are used to understand and decipher the cell/matrix interface at all its levels of complexity and dimensional scale, from the smallest molecule (matrikine) to gigantic structures (collagen and/or elastin fibers). The MIME team's approach is based on a set of multi-scale visualization and analysis methods, both qualitative and quantitative, including original methods developed specifically.

Communications orales

Comparison between rat tail tendon and Wharton's jelly extracellular matrix hydrogels as biomaterial

Anaïs Lavrand[a], Lorinne Adam[a], Flora Lemaire[a], Alexandre Berquand[a], Cédric Mauprivez[a,b], Esteban Brenet[a,b], Halima Kerdjoudj[a*]

[a] *Université Reims Champagne Ardenne, Reims, France*
[b] *Centre Hospitalier Universitaire de Reims, Reims, France*

* halima.kerdjoudj@univ-reims.fr

Keywords: Wharton's jelly, collagen, hydrogel

Abstract:

Extracellular matrix (ECM) hydrogels are the subject of growing interest in the field of biomaterials for their bioactive properties and their composition close to the organs. Rat tail tendon (RT) is used as a reference for ECM hydrogels and it is well described in literature. Wharton jelly (WJ) is a part of the umbilical cord that surrounds the blood vessels, serving to protect them. The ECM of this tissue is very rich in collagen and glycosaminoglycans (GAGs) and demonstrated regenerative properties [1]. By various technics we explored the mechanical and biological properties of ECM hydrogels of RT and WJ, and their potential use as biomaterials.

ECMs were extracted according to Freyte's method [2], and hydrogels were obtained in physiological conditions (pH 7.4, 37°C). Physico-chemical and in vitro and in vivo Biological characterizations of these hydrogels were conducted.

Through circular dichroism and SDS-PAGE electrophoresis, we demonstrated no collagen denaturation after the tissue processing. Collagen quantification revealed similar concentration in both hydrogels, but WJ-ECM showed higher concentrations of GAGs (2-times, $p<0.001$). After gelation, the WJ-ECM hydrogel was translucid achieving an average transmittance at 650 nm of 90% vs. 40% for RT-ECM hydrogel. Although in vitro fibrillogenesis of both hydrogels was of the same magnitude (> 80%), the atomic force microscopy revealed that the diameter of the collagen fibers in WJ-ECM hydrogel was 3-times smaller than in RT-ECM hydrogel ($p<0.01$), suggesting that the smaller well-dispersed collagen fibers in the WJ-ECM form a light-passible nanoscale network. The difference between the fibers diameter could be attributed to the presence of collagen type I and III in WJ-ECM whereas RT-ECM is mainly composed by collagen type I. Thanks to molecular modelisation, we found that also water organization around collagen I and III impacts fibrils autoassembly. In terms of mechanical properties, both hydrogels are soft and not easy to handle. The maximum G' was 137±18 Pa for WJ-ECM hydrogel and 166±9 Pa for RT-ECM hydrogel ($p<0.001$). Hydrogels are also subject to syneresis, that means water loss, with and without physical stimulation with more importance for RT-ECM hydrogels.

Both hydrogels were no cytotoxic. After tests on immune cells, neutrophils were less activated in the presence of WJ-ECM hydrogels compared to RT-ECM shown by release of ROS that was reduced by half ($p=0.05$). In a subcutaneous rat model, no foreign body reaction was observed after 56 days of implantation. Hydrogels have a limited porous structure, which can restrict cellular activity and molecular diffusion. To address this, ECM hydrogels were freeze-dried to produce porous sponges. WJ and RT-ECM sponges exhibited a loose structure with thicknesses of 2.03±0.2 and 1.3±0.1 mm, respectively ($p<0.01$) and both had around 95% of porosity. The WJ-ECM sponge absorbed twice as much water as RT-ECM sponge ($p=0.05$), likely due to the higher GAGs content and thinner collagen fibers, which provide a greater contact area. Collagen sponges are usually used for haemostatic properties and our WJ-ECM sponge showed a blood coagulation index twice as low as RT-ECM forms ($p<0.001$) and a clot with higher mechanical properties.

All these results suggest that WJ-ECM forms have promising potential as biomaterials for medical applications. Hydrogels potential use as bioink for bioprinting is also being investigated.

References:

[1]: M. Dubus et al., *Biomedicines*, 2022, **10**, 227
[2]: L. Saldin et al., *Acta biomaterialia*, 2017, **49**, 1

Engineering a regenerative mesenchyme for age-related diseases therapy : example of periodontitis

Jeanne Minvielle Moncla [ab], Marwan Puaud[b], Christophe Guissard[ac], Pilar Oreja-Fuentes[c], Louis Casteilla[a], Laurent Malaquin[b], Laurence Vaysse[a], Philippe Kemoun[ac], Julie Foncy[b]

[a] *RESTORE – A geroscience and rejuvenation center – Toulouse, France*
[b] *LAAS: research laboratory specialising in systems analysis and architecture – Toulouse, France*
[c] *Oral Medecine Department and CHU of Toulouse – Toulouse, France*

jeanne.minvielle-moncla@inserm.fr, mpuaud@laas.fr, christophe.guissard@inserm.fr, maria-del-pilar.oreja-fuentes@inserm.fr, louis.casteilla@inserm.fr, lmalaquin@inserm.fr, laurence.vaysse@inserm.fr, philippe.kemoun@inserm.fr, jfoncy@laas.fr

Keywords: regeneration, mesenchymal stromal cells (MSC), age related diseases, advanced medicinal therapeutic product (ATMP)

Abstract:
This project aims to create a new ATMP, to address age-related chronic inflammatory diseases, with a focus on periodontitis. It is a condition leading to the destruction of the periodontium (tooth-supporting tissue, including gingiva) that has reached a therapeutic impasse. Although MSCs, key-actors of mesenchyme involved in repair processes, demonstrated some benefits in periodontal regeneration when injected as cellular suspension, but these effects are often temporary, leading to unpredictable outcomes. Building on the team's previous work, we are developing a tissue engineering strategy to design microenvironment by mimicking the extracellular matrix using hydrogels. It provides essential biomechanical support and guidance for MSCs toward a regenerative phenotype. For this purpose, we have designed a comprehensive analysis consisting of an *in vitro* phase followed by an *in vivo* phase, allowing us to assess regeneration. To this end, we realised a multimodal and a multitemporal monitoring over time from the manufacture to the implantation using a reverse engineering approach to modulate and characterize the object during the culture phase *in vitro*, a strategy relevant to identify mesenchymal-supportive regenerative pathways specific readouts. Thus, we developed a cylindrical shape we called "cylindroid", which enables the investigation of cell-cell and cell-matrix interactions, critical in tissue repair. The iterative back-and-forth between *in vitro* and *in vivo* phases progressively highlighted the optimal carrier biomaterial architectural parameters to enhance the post-implantation tissue response. After encapsulation, we demonstrated that MSCs pull and remodel the material over time, eventually transforming the cylindrical shape, in soft hydrogels, while harder hydrogels retain their cylindrical shape, and cell behaviour differs markedly. Overall *in vivo* outcomes highlighted the inflammatory response around the ATMP, its resorption, and the survival of the implanted MSCs depend on the hydrogel composition and the culture duration before implantation. Preliminary data suggest a better long-term gingival repair when MSCs were grafted in hydrogel as compared to control groups.

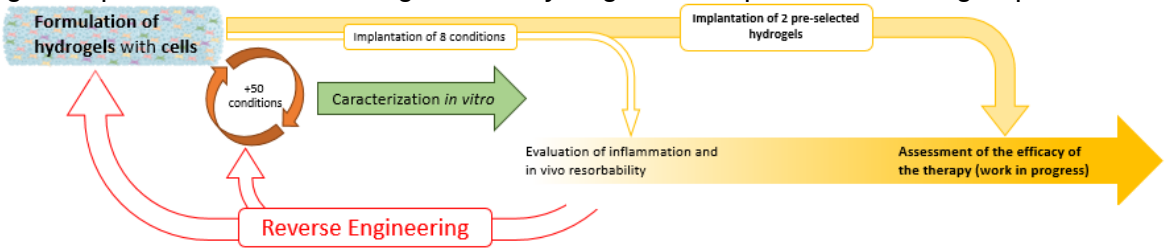


***Figure** – Project strategy based on reverse engineering approach explanation*

References:

Han, Yu, Xuezhou Li, Yanbo Zhang, Yuping Han, Fei Chang, et Jianxun Ding. 2019. « Mesenchymal Stem Cells for Regenerative Medicine ». Cells 8(8):886.

Kemoun, Ph, I. Ader, V. Planat-Benard, C. Dray, N. Fazilleau, P. Monsarrat, B. Cousin, J. Paupert, M. Ousset, A. Lorsignol, I. Raymond-Letron, B. Vellas, P. Valet, T. Kirkwood, J. Beard, L. Pénicaud, et L. Casteilla. 2022. « A Gerophysiology Perspective on Healthy Ageing ». Ageing Research Reviews 73:101537.

Li, Jianjun, Yufan Liu, Yijie Zhang, Bin Yao, Enhejirigala, Zhao Li, Wei Song, Yuzhen Wang, Xianlan Duan, Xingyu Yuan, Xiaobing Fu, et Sha Huang. 2021. « Biophysical and Biochemical Cues of Biomaterials Guide Mesenchymal Stem Cell Behaviors ». Frontiers in Cell and Developmental Biology 9:640388.

Escudero, Mélanie, Laurence Vaysse, Gozde Eke, Marion Peyrou, Francesc Villarroya, Sophie Bonnel, Yannick Jeanson, Louisa Boyer, Christophe Vieu, Benoit Chaput, Xi Yao, Frédéric Deschaseaux, Mélissa Parny, Isabelle Raymond-Letron, Christian Dani, Audrey Carrière, Laurent Malaquin, et Louis Casteilla. 2023. « Scalable Generation of Pre-Vascularized and Functional Human Beige Adipose Organoids ». *Advanced Science* 10(31):2301499.

Structural characterization and functional role of glycosaminoglycans from platelet-rich plasma in osteoarthritis chondroprotection

SAADAN Chayma[1,3], CACHEN Laurie[1,2], PINCHEMEL Marion[1,2], LAMMARI Yasmine[1,2], FLAGEOLLET Camille[1,3], BOEZENNEC Bruno[3], EYMARD Florent[1,2] et ALBANESE Patricia[1].

[1] *Laboratoire Glycobiologie, Croissance, Réparation, et Régénération tissulaire (Gly-CRRET), Université Paris-Est Créteil (UPEC), F-94000*
[2] *Assistance Publique-Hopitaux de Paris (AP-HP), Service de Rhumatologie, HôpitalHenriMondor, F-94000 Créteil*
[3] *RegenLab, F-91940 Les Ulis.*

csaadan@gmail.com

Keywords: Osteoarthritis, Extracellular Matrix, Glycosaminoglycans, Platelet-Rich Plasma, Heparan Sulfates

Abstract:

Purpose: Osteoarthritis (OA) is the most common joint disease, affecting millions of people worldwide, and closely associated with aging. It is characterized by cartilage extracellular matrix (ECM) degradation and low-grade inflammation of the synovial membrane. Current OA treatments provide symptomatic relief but fail to halt cartilage degradation. Among therapeutic approaches, intra-articular platelet-rich plasma (PRP) injections in the joint mixed with synovial fluid (SF), represent an autologous option. PRP's benefits are primarily attributed to its high concentration of growth factors, most of which are heparin-binding proteins (HBP). Our hypothesis is that PRP's therapeutic effects may be mediated by its glycosaminoglycan (GAG) composition and their interactions with HBP also. GAG are linear and sulfated polysaccharide chain based on disaccharide unit repetitions with very complex structures, that are covalently bound to core proteins to form proteoglycans (PG). Among GAG, Sulfated pattern of Heparan Sulfate (HS) are tightly regulated during physio-pathological processes[1] and are directly involved in their ability to interact with HBP (growth factors, enzymes, and cytokines) and regulate their biological activities. We recently demonstrated that despite their rarity in joint ECM, HS sulfation was increased in OA human cartilages and SF, associated to modifications of HBP binding and biological effects on chondrocytes and synoviocytes phenotypes[2]. Our main objective is to demonstrate that PRP contains GAG and compare structural and functional features of GAG from healthy or OA PRP to GAG from SF.

Methods: PRP were obtained from Blood samples from healthy (CT) donors (EFS agreement) and OA patient (Henri Mondor's Rheumatology Department HMRD) according to RegenLab® kit. SF samples (from HMRD) were characterized according to their growth factors and cytokines profiles using ELISA kits. Total GAG form PRP and SF were extracted, quantified via DMMB assay and HS concentrations measured after enzymatic digestions. GAG lengths were analyzed by gel migration profiles and HBP affinity determined using a specific ELISA competitive binding assay. Primary murine chondrocytes were stimulated with SF and PRP (CT and OA), GAG from SF and PRP, enzymatically GAG-depleted SF and PRP (CT and OA), and IL1β (1 ng/mL). The expression levels of ECM-degrading enzymes (MMP3, MMP13) were compared across conditions.

Results: We collected the PRP from 45 healthy donors and 21 OA patients. IL8 and FGF2 concentrations were increased in OA-PRP as compared to CT-PRP. The total GAG concentration was increased in OA PRP (7,4±4,3 µg/mL of PRP) as compared to CT PRP (2,8±1,5 µg/mL of PRP). No significant correlation was found between GAG concentrations in PRP and demographic data (age and sex). The concentrations of HS and CS were significantly different between OA and CT PRP, as well as between PRP and SF. The migration profiles of GAG from PRP revealed size differences. Binding affinities for FGF2 and VEGF tends to be increased for GAG from OA PRP as compared to CT-PRP and was 20 times stronger toward pleiotrophin for GAG from OA-PRP than CT-PRP. Chondrocyte stimulation with PRP (CT and OA) showed a chondroprotective effect, evidenced by a decrease in MMP3 and MMP13 expression compared to chondrocytes stimulated with IL1β, and negative controls. Conversely, stimulation with GAG-depleted PRP increased MMP3 and MMP13 expression.

Conclusion: This study demonstrates for the first time the presence of GAG in PRP, with significant differences between CT and OA samples in their concentrations, structural features and functional properties. Finally we show that presence of GAG in PRP is critical for its chondroprotective effects on murine chondrocytes. This suggest that controlling the structural and functional integrity of GAG in the joint is challenging to optimized new therapeutic strategies.

References:

[1] Ouidja *et al.*, *Essays Biochem.*, 2024
[2] Shamdani *et al.*, *Arthritis Research and Therapy*, 2020

TAX2 peptide as a promising antithrombotic agent: A mechanistic approach

Gaëlle Rousselet *[a], Christophe Schneider [a], Alexandre Raoul [b], Olivier Bocquet [a], Adeline Porcherie [b], Véronique Regnault [c], Albin Jeanne [b], Pascal Maurice [#a], Stéphane Dedieu [#a]

[a] *UMR CNRS 7369 Matrice Extracellulaire et Dynamique Cellulaire (MEDyC), Université de Reims Champagne-Ardenne, Reims, France*

[b] *Apmonia Therapeutics SAS, Reims, France*

[c] *INSERM U1116 Défaillance Cardiovasculaire Aigüe et Chronique (DCAC), Université de Lorraine, Nancy, France*

* gaelle.rousselet@univ-reims.fr

Keywords: thrombospondin-1, CD47, antithrombotic, platelet

Abstract:

Cardiovascular diseases remain the leading cause of death worldwide with stroke and myocardial infarctions being the most frequent. In such pathologies, extracellular matrix (ECM) remodelling becomes abnormal leading to tissue stiffening, impaired function, and increased risk of complications at the heart and blood vessels levels. Platelets play a critical role in primary haemostasis by forming thrombi at sites of vascular injury but are also central to the pathogenesis of arterial thrombosis. Despite the efficacy of current antiplatelet therapies in preventing arterial thrombi, a major limitation is the increased risk of bleeding. This has driven the search for alternative therapeutic targets that mitigate thrombosis without compromising haemostasis. Thrombospondin-1 (TSP-1), a matricellular glycoprotein released from alpha-granules during platelet activation, promotes thrombosis via CD47-dependent inhibition of the nitric oxide (NO)/cGMP/PKG signalling pathway. Selective disruption of the TSP-1/CD47 axis therefore represents a promising therapeutic opportunity. In this context, we developed TAX2, a cyclic dodecapeptide acting as an orthosteric antagonist of TSP-1/CD47 binding [1]. Previous studies demonstrated the antithrombotic properties of TAX2 *in vitro*, inhibiting platelet aggregation and adhesion, as well as *in vivo*, reducing carotid artery thrombosis in mice and rats models, without prolonging bleeding time [2,3]. The current study aims to elucidate the mechanism of action of TAX2 in the modulation of platelet function and the prevention of thrombosis.

Human platelets from healthy donors were collected and isolated by centrifugation, then subjected to agonist-induced activation. Platelet aggregation, intracellular signalling (SDS-PAGE and western blot), production of secondary mediators (luminescent and colorimetric assays), surface activation markers (flow cytometry) were assessed under TAX2 treatment.

Disruption of the TSP-1/CD47 interaction by TAX2 significantly reduced platelet aggregation in response to collagen (1-6 µg/mL) or TRAP-6 (5-30 µM) by 58.3 ± 7.0% and 36.1 ± 2.6%, respectively, compared to a control peptide. Granule secretion was only moderately affected. Interestingly, the combination of TAX2 with 10 nM of tirofiban, an antagonist of the $\alpha_{IIb}\beta_3$ integrin, achieves an inhibition comparable to that obtained with 25 or 50 nM of tirofiban alone. TAX2 also increases intracellular cGMP levels (by 2.5-fold), independently of CD36, as assessed using a CD36 blocking antibody (FA6-152). Signalling pathway analyses reveal that TAX2 markedly reduced phosphorylation of key signalling proteins upon platelet activation, including LAT (-48.9 ± 8.7%), PI3K (-54.9 ± 5.42%), AKT (-67.3 ± 6.8%) and SRC (-46.6 ± 7.4%). This led to reduced $\alpha_{IIb}\beta_3$ integrin activation and decreased fibrinogen binding to platelets. Moreover, TAX2 also diminished the platelet-dependent thrombin generation by 50%.

Our findings, which integrate results from multiple preclinical models in mice, rats, and dogs [3] along with mechanistic data, support the therapeutic potential of TAX2 as an antithrombotic drug and document its mode of action. Its favourable safety and toxicological profile, along with potential combination with existing antiplatelet drugs, paves the way for novel strategies in thrombosis management with reduced bleeding risk.

References:

[1]: A. Jeanne; T. Sarazin; M. Charlé; C. Moali; C. Fichel; C. Boulagnon-Rombi; M. Callewaert; M.-C. Andry; E. Diesis; F. Delolme; D. Rioult; and S. Dedieu, *Cancers*, 2021, **13**, 5006.

[2]: A. Jeanne; T. Sarazin; M. Charlé; C. Kawecki; A. Kauskot; T. Hedtke; C.E.H. Schmelzer; L. Martiny; P. Maurice; and S. Dedieu, *Arteriosclerosis, Thrombosis, and Vascular Biology*, 2021, **41**, 1433–1447.

[3]: A. Raoul; A. Henry; A. Jeanne; S. Dedieu; P. Maurice; A. Moniot; and A. Porcherie, *TAX2 peptide as a promising antithrombotic agent: A multispecies functional approach*, Abstract SFBMEc, 2025.

TAX2 peptide as a promising antithrombotic agent: A multispecies functional approach

Alexandre RAOUL[a*], Aubéri HENRY[a], Albin JEANNE[a], Stéphane DEDIEU[b], Pascal MAURICE[b], Aurélie MONIOT[a], Adeline PORCHERIE[a]

[a] *Apmonia Therapeutics SAS, Reims, France*
[b] *CNRS UMR 7369 MEDyC, Reims Champagne-Ardenne University, France*

* a.raoul@apmonia-therapeutics.com

Keywords: TAX2, Thrombosis, Platelet aggregation, Thrombospondin-1.

Abstract:
Thrombotic cardiovascular diseases remain the leading cause of death in industrialized countries. Although current antithrombotic therapies are effective, their use is limited by a significant risk of bleeding. Platelets play a crucial role in haemostasis and thrombosis, becoming activated upon vascular injury through interactions with matrix proteins and soluble agonists. This activation results in platelet granule secretion, including the release of thrombospondin-1 (TSP-1), a matricellular glycoprotein stored in the α-granules. TSP-1 interacts with multiple platelet receptors, such as integrins and CD47, thereby enhancing platelet activation and negatively regulating the NO–cGMP–PKG pathway, which amplifies thrombus formation. TAX2 peptide selectively antagonizes TSP-1 binding to CD47 [1], significantly reducing collagen-induced aggregation of human platelets and phosphorylation of key signalling proteins upon platelet activation [2]. In murine models of arterial thrombosis, TAX2 peptide delays thrombus formation in a same extent to mice lacking TSP-1 [3], thus ensuring target specificity. Notably, TAX2 peptide does not prolong bleeding time, underscoring its potential as a novel, safe antithrombotic therapy. While TAX2 was found to be well tolerated in rats and dogs during toxicology studies, we here demonstrate TAX2 pharmacological activity in these two species.

Platelet aggregation assays were performed using washed platelets isolated from canine or rat whole blood and incubated with either control (Tyrode's buffer) or TAX2 peptide at 400 and 800 µM. Samples were stimulated under stirring conditions in the presence of platelet agonists, i.e. collagen or adenosine 5'-diphosphate (ADP), in a light transmission aggregometer. The antithrombotic effect of TAX2 was evaluated in Sprague Dawley rats using a chemically induced carotid thrombosis model. Briefly, the carotid artery was surgically exposed and TAX2 peptide was administered intravenously. A ferric chloride ($FeCl_3$) patch was used to induce vessel injury, and the time to complete occlusion was measured using an ultrasound probe (VisualSonics).
TAX2 peptide inhibited canine platelet aggregation induced by collagen and ADP, in a dose-dependent manner. TAX2 peptide also demonstrated a significant dose-dependent inhibitory effect on rat platelet aggregation after stimulation with 20 µg/mL collagen. *In vivo*, TAX2 peptide (30 and 100 mg/kg) exhibited an antithrombotic effect in rats with a magnitude and efficacy similar to those previously observed in a comparable mouse model [3].

Our results, combining elucidation of TAX2 mechanism of action [2] and preclinical evaluation in mice, rats and dogs, confirm that TAX2 peptide is a promising antithrombotic therapeutic strategy, with a safety advantage over other anti-aggregant drugs, while displaying similar pharmacological activity in both rodent and non-rodent species. TAX2 peptide could enable more patients to benefit from a treatment that reduces the risk of occurrence or recurrence of cardiovascular events. To further strengthen these preclinical data, *in vivo* experiments in atherothrombosis and humanized mouse models are currently being conducted.

References:

[1]: A. Jeanne; T. Sarazin; M. Charlé; C. Moali; C. Fichel; C. Boulagnon-Rombi; M. Callewaert; M.-C. Andry; E. Diesis; F. Delolme; D. Rioult; and S. Dedieu, *Cancers*, 2021, **13**, 5006.

[2]: G. Rousselet; C. Schneider; A. Raoul; O. Bocquet; A. Porcherie; V. Regnault; A. Jeanne; P. Maurice; and S. Dedieu, *TAX2 peptide as a promising antithrombotic agent: A mechanistic approach*, Abstract SFBMec, 2025.

[3]: A. Jeanne; T. Sarazin; M. Charlé; C. Kawecki; A. Kauskot; T. Hedtke; C.E.H. Schmelzer; L. Martiny; P. Maurice; and S. Dedieu, *Towards the Therapeutic Use of Thrombospondin-1/CD47 Targeting TAX2 Peptide as an Antithrombotic Agent*, Arteriosclerosis, Thrombosis, and Vascular Biology, 2021, ***41***, 1433–1447.

Posters

Deciphering fibrin hydrogel proteolysis by dental pulp mesenchymal stem cells highlights the role of intrinsically disordered regions

Sean Thierry [a], Marjorie Faure [a], Lisa Reiniche [a], Marianne Leveque [a], Marielle Pasdeloup [a], Jean-Christophe Farges [a], Maxime Ducret [a] and Mourad Bekhouche *[a]

[a] *LBTI UMR-CNRS 5305 / Université Lyon 1, Lyon, France*

* mourad.bekhouche@univ-lyon1.fr

Keywords: fibrin hydrogel, metalloproteases, dental pulp, proteomics

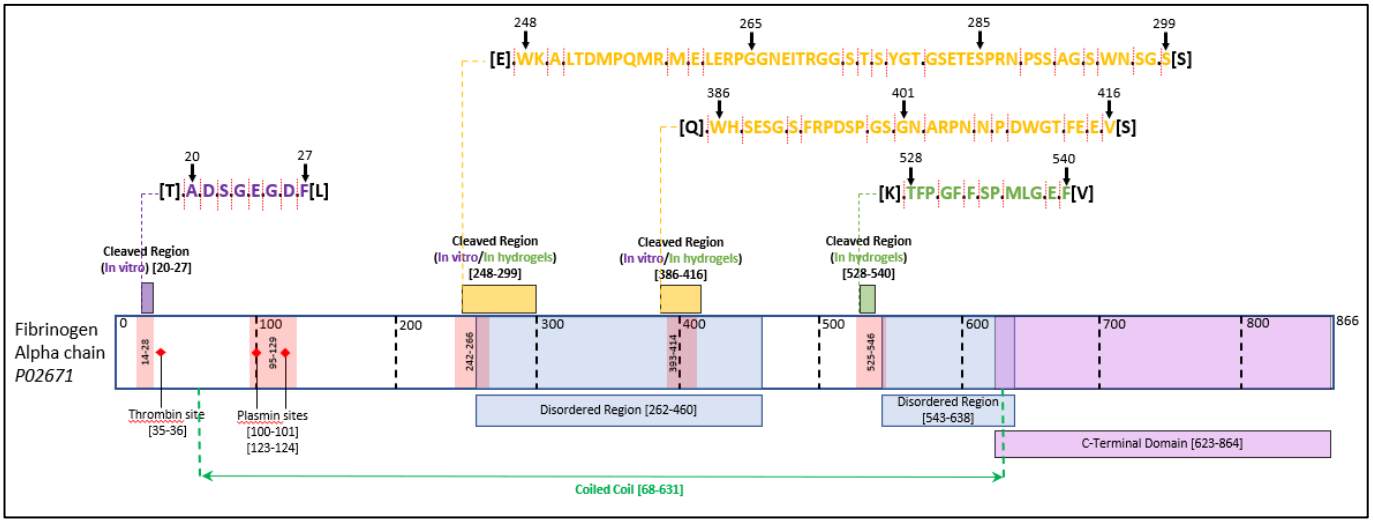
Abstract:

Background. Fibrin-based hydrogel seeded with dental pulp mesenchymal stem cells (DP-MSC) is proposed as alternative strategy of treatment for tooth devitalization [1]. However, this approach fails to regenerate a fully functional dental pulp and rather leads to fibrotic scarring or mineralized tissue formation due to uncontrolled fibrin degradation [2]. Previous omics approaches to investigate the mechanisms initiated by DP-MSCs in fibrin hydrogel show the overexpression of several MMPs including MMP3 [3]. In this work we investigated fibrin proteolysis by DP-MSCs and MMP3.

Materials & Methods. Fibrin hydrogel proteolysis by DP-MSCs or recombinant human MMP3 was investigated by immunoblot against the fibrinogen alpha chain. The precise cleavage sites were identified by degradomics. Peptides corresponding to selected cleavage sites were chemically produced and used as competitive inhibitor for MMP3 to evaluate the order of cleavage.

Results. Fibrin degradation products are observed after 2 days of culture. Degradomic experiments identified several cleavage sites within fibrinogen alpha chain (FGA) in addition to those already reported in the literature for thrombin and plasmin. Three different area preferentially cleaved are identified at positions 242-266, 393-414 and 525-546. The cleavage site specificity analysis reveals the abundance of glycine and serine amino acids located in intrinsically disordered regions of FGA. Kinetic experiments, using these cleavage areas as competitive inhibitors, lead to IC50 values of 29.9 µM, 163 µM and 1.8 mM, respectively for the peptides 525-546, 393-414 and 242-266.

Conclusions. This work points out the crucial role of the fibrinogen alpha-chain in fibrin hydrogel proteolysis by DP-MSCs and MMP3, and suggests an ordered mechanism. These data pave the way to new peptide-based strategies to monitor and control tissue regeneration.



Fibrinogen alpha chain degradation by dental pulp cells.

References:

[1] Ducret M, Costantini A, Gobert S, Farges JC, Bekhouche M. *European Cells and Materials,* 2021, **41**, 1-14.
[2] Richert R, Ducret M, Alliot-Licht B, Bekhouche M, Gobert S & Farges J-C. *International Endodontic Journal (IEJ),* 2022, **55** Suppl 1, 14-36. doi: 10.1111/iej.13683
[3] Tong D, Gobert S, Reuzeau A, Farges J.-C., Leveque M, Bolon M, Costantini A, Pasdeloup M, Lafont J, Ducret M, Bekhouche M. *Heliyon,* 2024, **10**(13):e32891.

Formulation of a new composite biomaterial for bone tissue engineering

Hamza Danguir[a,b], Khalil El Mabrouk[b], Maxime Ducret[a], Meriame Bricha[b], Mourad Bekhouche[a]

[a] Université Claude Bernard Lyon 1 – Faculté des sciences – University of Lyon, CNRS UMR5305, Tissue Biology and Therapeutic Engineering Laboratory (LBTI), F-69367 Lyon, France – France
[b] Université Euro-méditerranéenne de Fès – Morocco

Keywords: bone – bioglass – fibrin – tissue regeneration

Abstract:

PURPOSE: For bone regeneration, surgical grafts come with many risks, such as morbidity, graft rejection by the immunity system, and with an infection rate increased by 3.05% [1]. With such dangerous drawbacks, new alternatives must be studied to regenerate bone without damaging the host. Our objective is to develop new cellularized composite biomaterials, with fibrin as a scaffold, and bioglass in the hydrogel, for bone regeneration. Fibrin is a temporary natural extracellular matrix for tissue repair [2] while bioactive glass is a promising material used for tissue mineralization in bone and dental tissue engineering [3] [4]. This work aims at combining new bioglass composition to fibrin hydrogel to produce a biocompatible extracellular matrix adapted to the repair of osteodental tissues, capable of enhancing osteogenic signals and mineralization.

METHODS: New bioglass compositions, with osteogenic and antibacterial properties, were synthesized with sol-gel method, by the combination of various ions (Zn^{+2}, Sr^{2+}, Na^{+}, PO_4^{3-}),. The structural and chemical properties of the powders were characterized using X-Ray Diffraction (XRD), Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared Spectroscopy (FTIR) for structural and chemical analysis, Brunauer–Emmett–Teller (BET) for textural analysis, and X-Ray Fluorescence (XRF) to determine the elemental composition of the materials. Bioactivity of the samples was tested on Simulated Body Fluid (SBF), with similar composition to human plasma, hence envaluating the bioglass‘ behavior in biological environment for periods from 6h to 14 days. The best bioglasses were selected to be combined with fibrin hydrogel, forming a composite. Dental pulp mesenchymal stem cells‘ (DP-MSCs) viability in these composite extracellular matrices was evaluated using Live/ Dead Assays after 24h and 48h, to determine the cytocompatible bioglass concentration . Moreover, calcium deposits were evaluated with Alizarin Red Staining (ARS) and alkaline phosphatase (ALP) activity was assessed on DP-MSCs grown for 14 days and 21 days.

RESULTS: Bioglass characterizations showed that those containing strontium are highly porous and bioactive, forming a crystal apatite layer, similar to bone hydroxyapatite in SBF. XRF confirmed the elemental composition of each bioglass, corresponding to the experimental values. Moreover, the same bioglasses showed satisfying cellular viability according to the norm ISO 10993-5 of medical devices, which is more than 70%, after 24 and 48 hours, unlike sodium rich bioglassses that can be toxic to cells. Strontium bioglasses showed the highest bioactivity, with more than 95% of live cells in the presence of 5-40 mg/mL bioglass. ALP activity was high in zinc and strontium bioglasses by comparison to the activity measured in sodium rich bioglasses. ARS also confirmed that strontium and zinc bioglasses induce calcium deposits on DP-MSCs, while sodium bioglasses are cytotoxic.

DISCUSSION: Sodium bioglasses can be toxic for cells, but a low concentration of sodium can be efficient. Moreover, strontium is a very bioactive ion, which can be useful for bone regeneration purposes. Bioglass physical and chemical characterization showed that all bioglasses are bioactive in SBF, but further investigations are needed to evaluate the apatite layer formation of the whole hydrogel as a functional matrix that supports the physical and biological roles of the extracellular matrix.

CONCLUSION: By combining two biomaterials, this work highlights the benefits of each material in the formation of an extracellular matrix for mineralized tissue repair. This liquid form of the fibrin before polymerization will facilitate targeting radicular and periodontal mineralized tissues difficult to access using solid biomaterials.

References:

[1]: F.-H. Lee; P.-C. Shen; I.-M. Jou; C.-Y. Li; and J. -L. Hsieh, *Medicine (Baltimore)*, 2015, 94, e2023
[2]: P. Dec, A. Modrzejewski; and A. Pawlik, *Int J. Mol. Sci.*, 2022, 24, 529
[3]: G. Kaur, O. P. Pandey; K. Singh; D. Homa; B. Scott; and G. Pickreel, *J. Biomed. Mater. Res. A*, 2014, 102, 254-274
[4]: M. Ducret; A. Constantini; S. Gobert-Gosse ; J.-C Farges; and M. Bekhouche, *Eur. Cell. Mater.*, 2021 ; 41 ; 1-14

Evaluation and modelling of vascular microstructures evolution during normal and pathological aging using synchrotron X-ray three-dimensional microtomography

Alexandra Fontaine[1], Dênnis Da Silva[1,2], Xiaowen Liang[1,2], Aicha Ben Zemzem[1], Laetitia Vanalderwiert[1], Béatrice Romier-Crouzet[1], Sébastien Blaise[1], Anne-Elisabeth Molza[1], Hua Wong[1], Jean-Charles Boisson[3], Edith Grall[4], Guillaume Dollé[5], Florian Blachère[6], Stéphanie Salmon[5], Stéphanie Baud[1], Luiz-Angelo Steffenel[3], Pierre Beauseroy[4], Timm Weitkamp[7], Nicolas Passat[2], Laurent Debelle*[1], Sébastien Almagro*[1]

[1] *Université de Reims Champagne-Ardenne, CNRS, UMR 7369 MEDyC, Reims, France.*
[2] *Université de Reims Champagne-Ardenne, UR 3804 CReSTIC, Reims, France.*
[3] *Université de Reims Champagne-Ardenne, UR 3690 LRC DIGIT, Reims, France.*
[4] *Université de Technologie de Troyes, LIST3N, Troyes, France.*
[5] *Université de Reims Champagne-Ardenne, CNRS, UMR 9008 LMR, Reims, France.*
[6] *Université de Technologie de Troyes, GAMMA3, Troyes, France.*
[7] *Synchrotron SOLEIL, Saint-Aubin, France.*

* laurent.debelle@univ-reims.fr, sebastien.almagro@univ-reims.fr

Keywords: Aortic wall, elastic lamellae, aging, synchrotron imaging

Abstract:
Vascular aging is characterized by slow, insidious, and asymptomatic alterations of vascular microstructures, such as the elastic lamellae. Nevertheless, the early events forecasting these alterations remain mostly undocumented.
To address this critical question, MEDyC uses synchrotron X-ray microcomputed imaging to capture the discrete and fine alterations occurring during the silent phases of the aging process in mouse in normal and pathological conditions. For a single aorta, tomographic volume images of 3948×3948×2048 voxels (voxel size 0.65 µm) are recorded in the thoracic to abdominal region of the aorta. Finding, extracting, and analysing these massive and information-rich data is a considerable challenge. Indeed, they contain a wide range of details at different scales, which induces semantic noise in addition to acquisition noise that may disturb the analysis.
We have developed a prototype of a fully automated segmentation approach, able to process the images by relying both on standard image processing paradigms and deep-learning strategies (Siamese networks).
We are now able to measure and compare intramural features in aorta from animals experiencing normal (C57Bl6J strain) or pathologic (*db/db* strain, diabetes) aging.
The results obtained so far show that diabetic mice have smoother elastic lamellae than normal mice at the same age. Lamellae from diabetic mice have lost 24.8% of their reserve length. This effect is consistent with the fact that diabetic individuals are hypertensive. Further, we observe a concomitant loss of the lattice-like filamentous structure we have discovered within elastic lamellae.
We are currently collecting more data from healthy and diabetic animals (2-24 months, n = 15 for each time point) with the objective of modelling and predicting the evolution of aortic features during aging (ANR MODELAGE project).

The glycosyltransferase β4GalT7: a new target in substrate reduction therapy in mucopolysaccharidoses?

Déborah HELLE[1], Anne ROBERT[1], Nick RAMALANJAONA[1], Christel VALENCIA-SCHMITT[2], Pascal VILLA[2], Sylvie FOURNEL-GIGLEUX[1], Sandrine GULBERTI*[1]

1 UMR 7365 Université de Lorraine-CNRS, IMoPA, Vandœuvre-Lès-Nancy, France

2 Plateforme de Chimie Biologie Intégrative de Strasbourg (PCBIS), UAR3286, Illkirch, France

** sandrine.gulberti@univ-lorraine.fr*

Keywords: glycosaminoglycans, mucopolysaccharidoses, glycosyltransferases

Abstract:

Mucopolysaccharidoses (MPS) are a group of inherited lysosomal storage diseases caused by the deficiency of enzymes involved in the degradation of glycosaminoglycans (GAG). Their abnormal accumulation in tissues leads to cellular damages and progressive multiple organ dysfunction. Enzyme Replacement Therapy (ERT) consisting in injection of functional recombinant enzyme shows limitations that are mainly due to the low tissue distribution of the enzyme. Substrate Reduction Therapy (SRT), using small molecules able to cross the blood brain barrier and inhibit GAG biosynthesis to reduce their accumulation in tissues, represents a suitable alternative in MPS treatment.

In this context, we propose a new approach of SRT by developing small hydrophobic molecules to specifically inhibit the β1,4-galactosyltransferase 7 (β4GalT7), a key enzyme involved in GAG biosynthesis initiation. This would aim to prevent GAG accumulation and reduce the symptoms of most MPS, including neurological effects. Using an *in vitro* high throughput assay to screen the Prestwick Chemical Library, we have identified a series of β4GalT7 inhibitory candidates. These hits have been further confirmed and tested *in cellulo* using dermal fibroblasts, for their capacity to inhibit GAG synthesis. Two of these hit candidates showed significant inhibitory effect on the glycosylation of decorin, a model proteoglycan in our cell assays. These compounds will be the starting point to search for potential inhibitory chemical families of analogues and hit optimization. The study of physicochemical and pharmacokinetic properties of β4GalT7 inhibitors will initiate ADME-Toxicity tests on the best identified molecules to provide non-toxic drug candidates.

We hope that this project will provide original molecules that could help treating both the somatic and neurologic symptoms of MPS, by reducing GAG biosynthesis. This will open avenues to new therapeutic strategies for MPS by positioning glycosyltransferases (like β4GalT7) as potential therapeutic targets. These pharmacological compounds could be used alone or in combination with other therapeutics to treat severe forms of MPS and contribute to the improvement of the quality of life of patients.

Project funded by the French Foundation of Rare Diseases

Effects of pulsed electric fields on structured collagen matrix derived from decellularized dermal sheets

Nicolas Mattei [a], Emma Barrère [a], Marie-Pierre Rols [a], Muriel Golzio [a], Jelena Kolosnjaj-Tabi [a*]

[a] *Institut de Pharmacologie et de Biologie Structurale, CNRS UMR 5089*
* Jelena.Kolosnjaj-Tabi@ipbs.fr

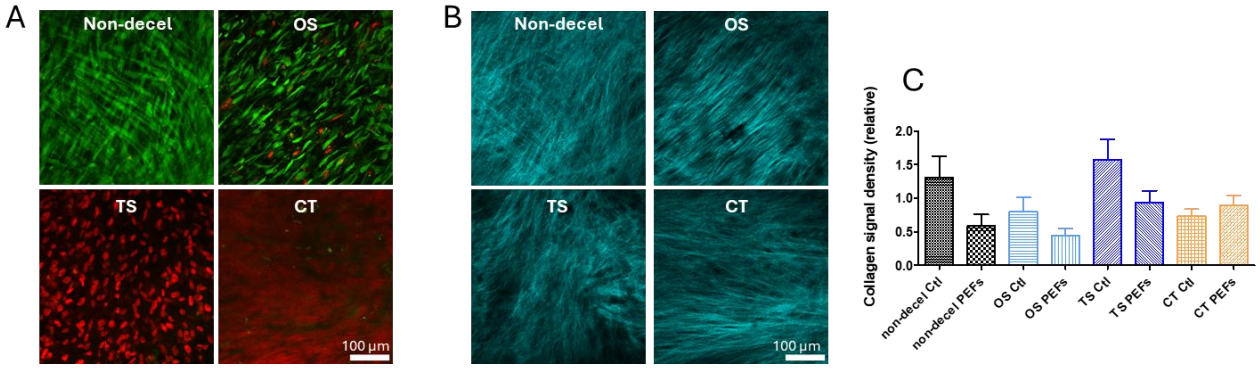
Keywords: decellularized engineered dermal sheet, collagen, electroporation, pulsed electric fields

Abstract:

Some solid tumors, such as pancreatic and liver tumors, are characterized by a dysregulation of the extracellular matrix (ECM). The ECM has important roles in cancer development, such as promoting tumor growth and metastasis [1]. Additionally, it acts as a barrier, hampering antitumoral drugs and immune cells penetration in the tumor [2]. Therefore, the tumoral ECM is nowadays considered as both, barrier and target for therapy improvement. Pharmacological approaches have been investigated but they lack specific regional onsets and cause deleterious systemic effects [3]. On the other hand, physical strategies have gained traction for their potential ECM remodeling capability. Among different strategies we focus on the application of pulsed electric fields (PEFs). This technique is used to induce a reversible or irreversible non-thermal permeabilization of cells, and has found multiple applications for the treatment of tumors [4]. It has been shown before that the application of PEFs can induce an increased activity of metalloproteinases secreted by fibroblasts, regulating the ECM [5]. Yet, there is a gap of knowledge in direct effects of the PEFs on the collagen matrix. Hence this work aims at filling this gap.

3D engineered dermal sheets comprising endogenously secreted collagen were produced as described before [6]. Next, they were decellularized through different methods: osmotic shock by incubation in water (OS), thermal shock by 3 cycles of freezing and thawing (TS), and chemical treatment by incubation in SDS and Triton X-100 (CT). After decellularizing treatments, the models' features were assessed using fluorophores (Figure A). Collagen in decellularized sheets was imaged by second harmonic generation with biphoton microscopy before and 10 minutes after classical irreversible PEF treatment (Figure B). IRE induced a reduction of collagen signal density in all sheets except the ones priorly treated by CT decellularization (Figure C).

This work is among the first to focus on the direct effects of PEFs on collagen after removing the cellular components and suggests that PEFs should be further explored to prime the extracellular collagen matrix.



Decellularized and non-decellularized dermal sheets submitted to pulsed electric fields. A: Cytoplasm of live cells stained with CFSE (green) and nuclei of dead cells stained with propidium iodide (red). B: Collagen fibers imaged with second harmonic generation microscopy. C: Collagen signal density of dermal sheets submitted to different decellularization methods, after pulse treatment (Ctl = 0 V/cm, PEFs = 2000 V/cm). Non-decel = non-decellularized, OS = Osmotic shock, TS = Thermal shock, CT = SDS + Triton X-100 treatment.

References:

[1]: T.R. Cox, *Nat Rev Cancer*, 2021, **21**, 217–238.
[2]: F. Schütze, F. Röhrig, S. Vorlová, S. Gätzner, A. Kuhn, S. Ergün, E. Henke, *Sci Rep*, 2015, **5**, 17576.
[3]: J. Kolosnjaj-Tabi, I. Marangon, A. Nicolas-Boluda, A.K.A Silva, F. Gazeau, *Pharmacol Res*, 2017, **126**, 123–137.
[4]: B. Geboers, H.J. Scheffer, P.M. Graybill, A.H. Ruarus, S. Nieuwenhuizen, R.S. Puijk, P.M. van den Tol, R.V. Davalos, B. Rubinsky, T.D. de Gruijl, D. Miklavčič, M.R. Meijerink, *Radiology*, 2020, **295**, 254–272.
[5]: S. Gouarderes, C. Ober, L. Doumard, J. Dandurand, P. Vicendo, I. Fourquaux, A. Golberg, V. Samouillan, L. Gibot, *J Invest Dermatol*, 2022, **142**, 1326-1337.e9.
[6]: L. Gibot, T. Galbraith, J. Bourland, A. Rogic, M. Skobe, F.A. Auger, *Nat Protoc*, 2017, **12**, 1077–1088

Development of a biphasic osteochondral model for joint tissue repair using extrusion-based 3D bioprinting of a natural composite hydrogel: a proof of concept

Marjorie Dufaud[1], Christophe Marquette[2], Christian Jorgensen[1], Danièle Noël[1], Emeline Perrier-Groult[1*]

1 IRMB, University of Montpellier, INSERM, Montpellier, France

2 3d.FAB, Univ Lyon, Université Lyon1, CNRS, INSA, CPE-Lyon, ICBMS, UMR 5246, Villeurbanne, France

* emeline.groult@inserm.fr

Keywords: Bioprinting; Osteochondral repair; MSCs

Abstract:

Extrusion-based 3D bioprinting is a promising technique to produce complex, three-dimensional (3D) structures that mimic the native extracellular matrix (ECM), which is pivotal for developing advanced tissue engineering solutions. Composite hydrogels, which are combinations of two or more distinct materials, can be used as a printing medium that offers several advantages over a single biomaterial due to their tunability, including improved mechanical properties and biocompatibility. These materials can be engineered to have specific physical, chemical, or biological properties, making them useful for a variety of applications. Extrusion-based 3D bioprinting of these hydrogels has generated high expectancy for joint tissue engineering, in particular for cartilage and bone repair. Here, we report the development of a 3D bioprinting process for the generation of biphasic 3D constructs for osteochondral repair of full-thickness joint lesions.

The bioink used in this study is based on a natural composite hydrogel developed at 3d.FAB Platform (Villeurbanne, France) comprising of gelatin (5%), alginate (2%), and fibrin (2%) loaded with murine mesenchymal stromal cells (mMSCs) expressing the differentiation factor BMP-2 under inducible conditions – a factor critically involved in both chondrogenesis and osteogenesis. Following printing using the BIO X 3D printer (CELLINK, Sweden) and crosslinking with a solution of transglutaminase/CaCl2/thrombin, the 3D bioprinted constructs were cultured individually under proliferation, chondrogenic or osteogenic conditions for a period of 28 days in order to optimize the culture conditions for enhanced cell differentiation.

Our findings showed: (i) high cell viability (>90%) of mMSCs across all conditions using the Live/Dead assay; (ii) sustained cell proliferation using the PrestoBlue Proliferation Assay; and (iii) cell differentiation towards either the chondrogenic or osteogenic lineages as shown by the up-regulation of specific differentiation markers by RT-qPCR. Cell differentiation was further analysed at the protein level using immunofluorescence labelling: type II collagen and aggrecan for cartilage; osteocalcin for subchondral bone. Finally, a biphasic construct comprising a cartilage compartment and a subchondral bone compartment was printed in an “all-in-one” strategy and analysed by immunofluorescence following different culture conditions. It showed the feasibility of printing complex cell-laden 3D constructs with different composition along the depth.

Overall, although some challenges remain to be overcome, 3D bioprinting holds great promise for the development of improved strategies for joint tissue engineering by transforming the treatment of joint injuries and diseases in the future.