Campagne 2020 Contrats Doctoraux Instituts/Initiatives

Proposition de Projet de Recherche Doctoral (PRD)

Appel à projet ISVI - Intitiative Sces du vivant ses interfaces 2020

Intitulé du Projet de Recherche Doctoral : Reconstruction par ingénierie tissulaire de la jonction myotendineuse

Directeur de Thèse porteur du projet (titulaire d'une HDR) :

NOM : **DUPREZ** Prénom : **Delphine**
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Unité de Recherche :
Intitulé : Laboratoire de Biologie du Développement (LBD)
Code *(ex. UMR xxxx)* : UMR7622

ED515-Complexité du Vivant

Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant :

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 1 étudiant inscription 2019 50% encadrement

Co-encadrant :

NOM : **LEGALLAIS** Prénom : **Cécile**
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Unité de Recherche :
Intitulé : laboratoire de Biomécanique et Bioingénierie (BMBI)
Code *(ex. UMR xxxx)* : UMR 7338

ED71 - Sciences pour l'ingénieur UTC
Ecole Doctorale de rattachement : Ou si ED non Alliance SU :

Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 6 co-encadrements à 50 %, dont 3 à soutenir avant été 2020, 1 en novembre 2020, 1 en 2021 et 1 en 2022.

Cotutelle internationale : ☒ Non ☐ Oui, précisez Pays et Université :

Description du projet de recherche doctoral *(en français ou en anglais)*

3 pages maximum – interligne simple – Ce texte sera diffusé en ligne

Détailler le contexte, l'objectif scientifique, la justification de l'approche scientifique ainsi que l'adéquation à l'initiative/l'Institut.
Le cas échéant, préciser le rôle de chaque encadrant ainsi que les compétences scientifiques apportées. Indiquer les publications/productions des encadrants en lien avec le projet.
Préciser le profil d'étudiant(e) recherché.

Summary

The myotendinous junction (MTJ) is a key component of the musculo-skeletal system as it links two major components, muscle and tendon. Tendon defects are major clinical challenge to orthopaedic surgery following injuries and during ageing. Tendon ruptures are frequently pinpointed at MTJ junction. Underlying mechanisms that mediate the exact composition and formation of MTJ are currently poorly understood. The objective of the project is to engineer an MTJ in order to adress the mechanisms underlying of MTJ formation. We will combine expertises on musculo-skeletal system development and tissue engineering to build an in vitro MTJ.

Scientific context

The myotendinous junction (MTJ) is a structure of specific interest in the musculo-skeletal system since it links two major components, muscle and tendon. Muscle contractions generate forces, which are transmitted to the skeleton via tendon to allow body movement. The MTJ structural integrity is critical for force transmission. On one side, skeletal muscle generates the mechanical forces. On the other side, tendon is an anisotropic and viscoelastic material able to resist to high tensile forces.

The ultrastructure of the MTJ is well described and was mostly explored using Transmission Electron Microscopy and Focused Ion Beam/Scanning Electron Microscopy. At this scale, the MTJ has been described as sarcoplasmic invaginations (ridge-like protrusions), which increase the contact surface between muscle and tendon. Multidirectional collagen fibers are observed on the tendon side, improving the anchorage between both tissues. A specific molecular signature of myonuclei at the MTJ has been recently identified by single-nucleus RNA-sequencing of myofibers in adult skeletal muscles in mice (Kim et al., 2020). Despite the well-described ultrastructure of MTJ, the current understanding of MTJ formation is poorly understood. The molecular and cellular interactions between muscle cells and tendon cells that lead to a functional MTJ are not fully understood (reviewed in Gaut and Duprez, 2016). We have identified markers displaying specific expression at the muscle and tendon interface, either in the muscle side or tendon side (DD, IBPS). We are currently exploring the molecular function the BMP signalling pathway in MTJ formation (DD, IBPS, ongoing work); the ligand being produced by the tendon and the BMP-responsive cells are muscle cells at the MTJ (Wang et al., 2010)

Literature on the in vitro reconstruction of the MJT is quite poor, probably because the molecular and cellular mechanisms leading to the formation of the MTJ have not yet been clearly established. Only few groups attempted to reconstruct the MTJ, using so-called scaffold-free self-organized tendon constructs (SOT), tissue engineered approaches or bioprinting. However, when subejcted to tensile tests, rupture was often observed on the muscle side (Ladd et al., 2011).

Scientific goals

In this project, we will adopt a bioinspired approach for the reconstruction of the MTJ. We will try to recapitulate the biological and mechanical cues that occur during MTJ formation and maturation instead of mimicking the "final" structure of MTJ. We propose to use our expertises on musculo-skeletal system development (DD, IBPS) and tissue engineering (CL, UTC) to build an in vitro MTJ. We will combine the biochemical and physical stimuli known to favour tendon and muscle formation in order to engineer a MTJ. The engineering of such MTJ would be beneficial for

fundamental studies to evaluate hypotheses regarding mechanisms potentially involved in the development of such a complex structure.

In the PhD program, we will engineer a complete structure, with 3 different compartments: muscle, tendon and MTJ. We will use the current knowkedge of in vivo development of tendon and skeletal muscle (DD, IBPS) but also our experiences in muscle and tendon cell culture conditions (DD, IBPS, CL, UTC) to investigate the different strategies to perform cell cultures with the bioreactors available in our laboratories, such as Bose Biodynamic, combined electrical and mechanical stimuli adapted from T6CellScale (CL, UTC) and the Flexcell machine (DD, IBPS).

For the project we will use mouse and rat primary cell cultures but also muscle and mesenchymal cell lines, which we already handle in each laboratory (Baudequin et al., 2015, 2017, 2019; Gaut et al., 2020). For the muscle cells, we will use C2C12 cells and primary muscle cells. We have already set the culture conditions that favour myoblast fusion. A PhD student, M. Beldjelali Labro (defense planned in september 2020, in UTC) designed PEG micropatterned electrospun PCL sheets covered with gold nanoparticles that favour myoblast fusion and promote myotube formation (Beldjilali-Labro et al., 2018). For tendon cells, we will use mesenchymal stem cells from rat ou mouse. We have already experienced the optimum 2D and 3D culture conditions, which favour tendon differentiation from mesenchymal stem cells (Guerquin et al., 2013, Gaut et al., 2016, 2020). From the mechanical point of view, we will play with the topography of the substrates in order to favour either myotube formation in muscle cultures or collagen synthesis in tenocyte cultures. When submitted to specific mechanical or electrical stimuli, we have already observed an increase in the number and size of myotubes (ongoing work, CL, UTC). We also have already demonstrated that the diameter of electrospun PCL fibers (Baudequin et al., 2017) and specific cycles of mechanical stretching favour cell alignment and tenomodulin production in the tendon bioengineered constructs (Garcia Garcia, PhD thesis 2019). In the culture set-up, we plan first to separate muscle and tendon compartments with a degradable phycical barrier (made of alginate gel for instance, easy to remove using alginate lyase). When cells will be engaged in their respective differentiation programs, we will remove the barrier located at the muscle/tendon interface and follow cell spreading, using GFP cells and/or cell trackers.

The tissue constructs will then be analyzed regarding both biological and mechanical outcomes. Biological outcomes will be analysed using histology, immunostaining and confocal microscopy, but also with genomic and proteomic assays. Mechanical consequences will be assessed with the measurement of the Young modulus obtained by strain/stress experiments and other mechanical parameters specific to visco-elastic tissues.

This project is in perfect adequation with the Interdisciplinary Initiative for Living Systems (I2LS) / Initiative Sciences du Vivant et ses Interfaces (ISVI). This project proposes a bioinspired, multiscale, integrative and time scale approach to reconstruct the MTJ. The in vitro reconstruction combining expertises in development biology, biomaterials and mechanotrasnduction will give access to features not easily accessible in vivo. It will pave the way to new advanced in vitro models that can be employed in organogenesis studies as well as in regeneration and repair.

The PhD supervision will be led by 2 co-directors:

Delphine DUPREZ (DR1 CNRS) in IBPS (UMR7622 CNRS-SU Laboratoire de Biologie du

développement / LBD) has expertise in organogenesis during development using animal models. Her major focus is to understand the mechanical and molecular signals that regulate the formation of the musculoskeletal system during development. She sees the development of the musculoskeletal system as an integrated process and focus on skeletal muscle and surrounding tissues such as tendon.

Cécile LEGALLAIS (DR1 CNRS) in UTC (UMR CNRS 7338 Biomechanics and Bioengineering/ INSIS). CL is specialized in tissue engineering, applied to the liver and more recently to the reconstruction of the musculoskeletal system. After a focus on bone tissue engineering, she developed in collaboration with DD strategies for tendon tissue engineering, and in the framework of Labex MSST (UTC) a larger project dealing with the reconstruction of the bone-tendon-muscle continuum.

Candidate's experience: either a MS degree in developmental and cell biology, with basic knowledge in biomaterials, or a MS degree in biomaterials with basic knowledge in cell biology, or an engineering diploma in biomechanics, biomaterials or bioengineering

Publications

Baudequin T, Bedoui F, Dufresne M, Paullier P, Legallais C. Tissue Eng Part A. 2015 Jun;21(11-12):1895-905.

Baudequin T, Gaut L, Mueller M, Huepkes A, Glasmacher B, Duprez D, Bedoui F, Legallais C. Materials (Basel). 2017 Dec 4;10(12).pii: E1387. PMID: 29207566.

Baudequin T., Legallais C, Bedoui F. Biotechnology J. 2019 10.1002/biot.201800358.

Beldjilali-Labro M., Garcia Garcia A., Farhat F., Bedoui F., Grosset J-F., Dufresne M., Legallais C. Materials 2018, 11(7), 1116 - 1165

Garcia Garcia A., Hébraud A., Duval, J.-L., Wittmer C., Gaut L., Duprez D., Egles C., Bedoui F., Schlatter G., Legallais C. ACS Biomaterials Science & Engineering 2018 4 (9), 3317-3326. DOI: 10.1021/acsbiomaterials.8b00521

Gaut L and Duprez D (2016) Tendon development and diseases. Wiley Interdiscip Rev Dev Biol. 2016 Jan-Feb;5(1):5-23. Review PMID: 26256998

Gaut L, Bonnin MA, Blavet C, Cacciapuoti I, Orpel M, Mericskay M, Duprez D. (2020) Biol Open. 2020 Jan 15. pii: bio.047928. PMID: 31941700

Gaut L, Robert N, Delalande A, Bonnin MA, Pichon C, Duprez D. (2016) PLoS One. 2016 Nov 7;11(11):e0166237. PMID: 27820865

Guerquin* MJ, Charvet* B, Nourissat G, Havis E, Ronsin O, Bonnin MA, Ruggiu M, Olivera I, Robert O, Lu Y, Kadler KE, Baumberger T, Doursounian L, Berenbaum F and Duprez D (2013) Journal of Clinical Investigation Aug;123(8):3564-76. PMID: 23863709

Kim M, Franke V, Brandt B, Spuler S, Akalin A, and Birchmeier C (2020) bioRxiv doi.org/10.1101/2020.04.14.041665

Ladd, M.R.; Lee, S.J.; Stitzel, J.D.; Atala, A.; Yoo, J.J. Biomaterials 2011, 32, 1549–1559

Wang, H., Noulet, F., Edom-Vovard, F., Tozer, S., Le Grand, F. and Duprez, D. (2010). Developmental Cell 18, 643-54. PMID: 2041277

Merci de nommer votre fichier pdf :
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à envoyer simultanément par e-mail à l'ED de rattachement et au programme :
cd_instituts_et_initiatives@listes.upmc.fr avant le 30 mars.