Coupling a bioartificial tendon/liver-on-chip to evaluate drug toxicity: a feasibility study

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INTRODUCTION

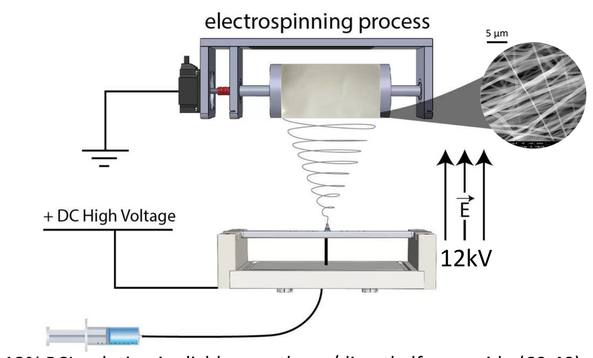
Some drugs cause harmful side effects for the tendons, which can range from tendinitis to total rupture. Some antibiotics from the fluoroquinolone family, statins or even corticosteroids are known to induce tendinopathy. The effects of these molecules, and often by metabolites produced in liver, are notably due to an alteration of collagen I, the main protein in the tendon extracellular matrix, and to tenocytes' dysfunction [1]. These side effects were not evidenced in preclinical studies, partly due to the lack of relevant models.

OBJECTIVE

We aim at developing a microphysiological system to evaluate iatrogenic effects on tendon by adapting the coculture model designed at UTC [2]. We plan to couple two compartments in a close loop microfluidic circuit, one containing a simplified *in vitro* engineered tendon and the other a biochip seeded with hepatocytes, in medium containing dexamethasone and atorvastatin.

IN VITRO BIOARTIFICIAL TENDON ENGINEERING

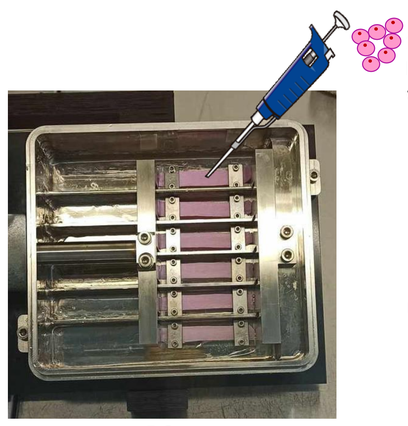
PCL (POLYCAPROLACTONE) ALIGNED FIBERS SCAFFOLD PREPARATION:



12% PCL solution in dichloromethane/dimethylformamide (60:40)



6 x 0,9 cm PCL pieces were clamped in T6 mechanoculture bioreactor (Cell scale)



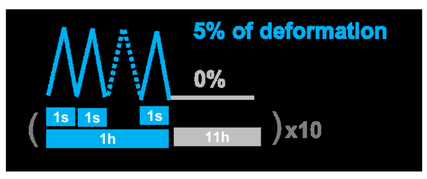
D0

180000 hBMMSC (human Bone Marrow Mesenchymal Stem Cells) were seeded on each scaffold

DMEM complete medium
+ ascorbic acid 50µg/mL
+ TGFβ1 5 ng/mL

Static culture
2 days @ 37°C 5%CO2

D2



Dynamic culture
5 days @ 37°C 5% CO2
Uniaxial stretch of 5% strain for 1h at 1 Hz followed by 11h of rest (2 cyles of stretching per day)

D7

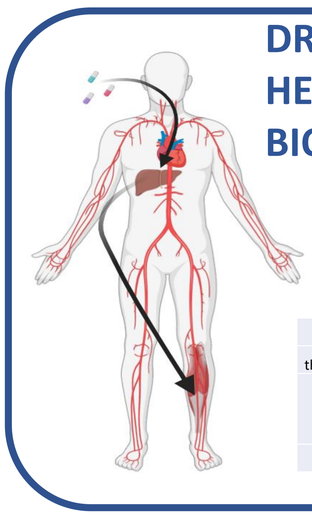
BIOARTIFICIAL TENDON EVALUATION

Collagen I , tenomodulin, and scleraxis immunodétection

Actin and nuclei staining

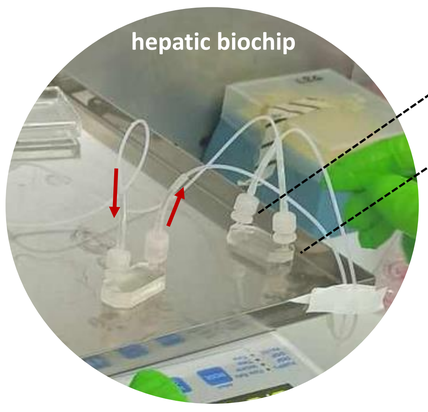
Hydroxyproline quantification

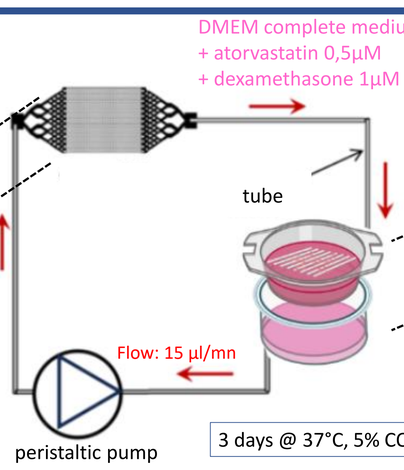
DRUG TESTING MODEL: HEPATIC BIOCHIP COUPLED TO BIOARTIFICIAL TENDON



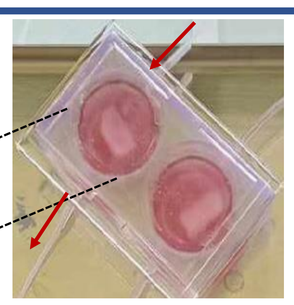
mimicking liver biotransformation with organ-on-chip technology

drug tested	dexamethasone	atorvastatin
therapeutic category	glucocorticoïd	antilipidemic
metabolites	6β-hydroxydexaméthasone	Ortho-hydroxyatorvastatine Para-hydroxyatorvastatine
bioavailability	80%	12%





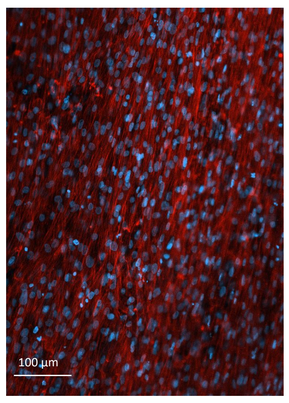
3 days @ 37°C, 5% CO2



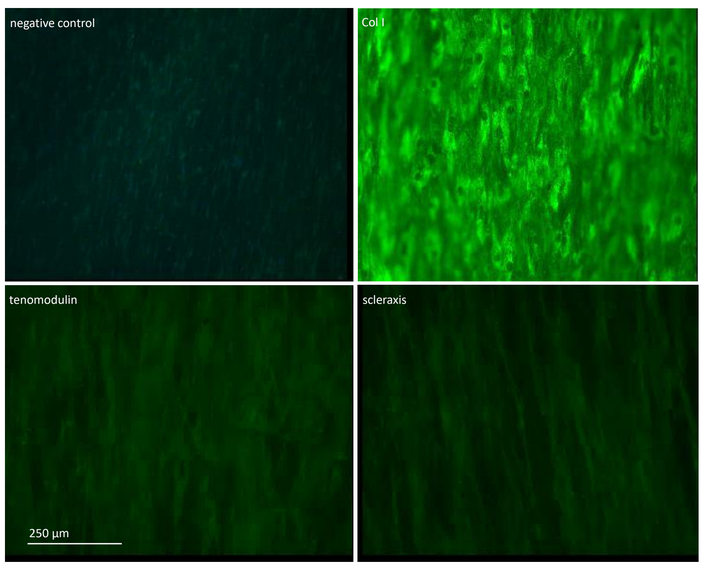
Bioartificial tendons were placed in culture inserts on microfluidic platform. A peristaltic pump allowed the medium circulation between tendons and liver biochips in a close loop circuit. As control, bioartificial tendons were incubated in culture inserts without flow.

Col I immunodetection, cell and nuclei morphologies observation by actin and DAPI staining respectively

Bioartificial tendon evaluation



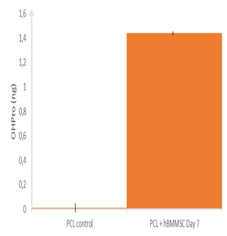
Actin staining showed that cells aligned along PCL fibers and nuclei adopted an elongated shape as expected for tenocytes (confocal microscopy).



hBMMSC cultured for 7 days on PCL scaffold under 5 days of mechanical stimulation expressed Col I and tended to differentiate into tendon as seen with tenomodulin and scleraxis markers (epifluorescence microscopy).

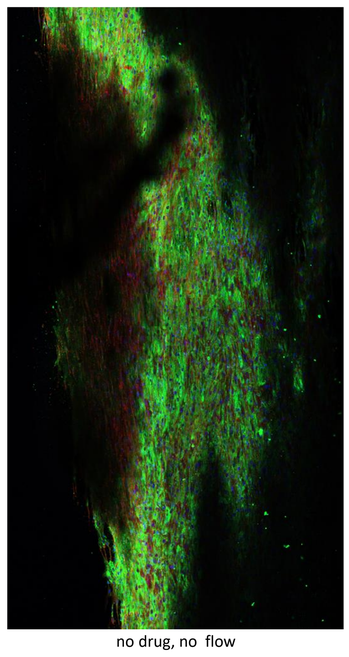
RESULTS

Hydroxyproline quantification

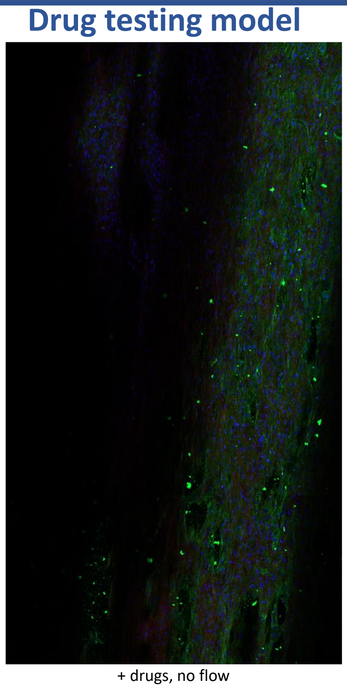


Collagen produced by hBMMSC after 7 days of culture on PCL scaffold was measured by hydroxyproline quantification kit, enabling the use of a quantitative method to analyse the effects of drugs on tenocytes functionality. (n=3)

Drug testing model



no drug, no flow



+ drugs, no flow



+ drugs, in coculture with hepatic biochip on microfluidic platform

Confocal microscopy analysis of Col I production on bioartificial tendons showed a decrease in Col I signal intensity when grown in the presence of atorvastatin in combination with dexamethasone. When bioartifical tendons were coupled to hepatic biochips we detected tears in the tissue (arrows), suggesting that hepatocytes could have transformed the drugs into metabolites more deleterious for the tendons.

CONCLUSION AND PERSPECTIVES

In these first experiments, we succeeded to develop a simplified engineered tendon by inducing hBMMSC differentiation on a PCL scaffold submitted to mechanical stress. These bioartificial tendons could be coupled with hepatocytes seeded biochips to mimick liver biotransformation of drugs. In this condition, we observed a decrease in collagen I expression and disruption of the tendon tissue, suggesting that drugs have been transformed by liver cells to form more harmful metabolites.
Nevertheless, further experiments need to be done to verify the reproducibility of the iatrogenic effects in our model and to evaluate hepatocytes behavior, biotransformation enzymes (CYP) activity and drug metabolites production. Furthermore, the presence of other collagens, such as Col III or Col XII should be studied.
Although they are very preliminary, our results are encouraging us to continue to develop a non-animal alternative method that integrates inter-organ crosstalk to evaluate drugs that could induce tendinopathy.

Références:
[1] Bolon, Toxicologic Pathology, 2017
[2] Madiedo-Podvrsan et al., Toxicology in Vitro, 2023

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