TRIPHASIC ELECTROSPUN SCAFFOLD WITH DYNAMIC CELLS COCULTURE FOR TISSUE ENGINEERING OF BONE-TENDON-MUSCLE JUNCTIONS.

Nicolas Rivoallan (1,2), Morgane Lagier (1), Timothée Baudequin (1), Marc Mueller (2), Pascale Vigneron (1), Rachid Jellali (1), Birgit Glasmacher (2), Cécile Legallais (1)

1. Université de technologie de Compiègne, CNRS, BMBI (Biomechanics and Bioengineering), Centre de recherche Royallieu - CS 60 319 - 60 203 Compiègne Cedex; 2. Institute for Multiphase Processes, Leibniz University Hannover, Hannover DE-30823, Germany.

Introduction

Microstructures in electrospun scaffolds are known to guide the differentiation of stem cells. [1-3]. In order to study the bone-tendon-muscle junctions, we propose to generate micrometric walls on a part of the aligned area of a formerly electrospun bi-phasic scaffold (honeycomb / aligned fibres). On this triphasic structure, C2C12 cells were located between the microwalls and cocultured in dynamic condition with C3H10T1/2 seeded on both the honeycombs and the aligned fibers.

Methods

The homemade collector combines patterned regions, gap spinning and regular electrospinning. Photolithography was used to microstructure wafers for the patterned regions and to add PEG walls of 50 µm width spaced of 500 µm on the scaffold. Solutions of 10 and 12%wt/v polycaprolactone were used to produce a two-layered scaffold, the first composed of beads-on-string fibers to increase the thickness of the honeycomb structure [4], the second composed of aligned fibers only (Fig. 1). Elastic moduli of each area of the material were determined using uniaxial tensile testing with video tracking. C3H10T1/2 and C2C12 cells were seeded at a density of 100 000 cells/cm² on the scaffolds without any differentiation factor for one week in static and dynamic conditions. Two programs of strain stimulation were tested. They consist every 11h in 3% and 5% of strain after 5 and 2 days of static culture respectively. The early bone differentiation was evaluated by ALP staining. Immunostaining was used to observe the bone, tendon and muscle fates of both cell lines. Moreover, the scaffolds and the cells were observed on SEM.

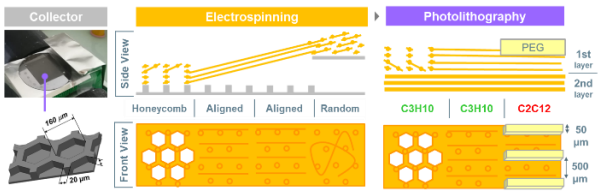

Figure 1: Schematic scaffold manufacture and coculture

Results

Micro walls were well added to obtain the triphasic scaffold. Those different areas showed significantly different mechanical properties with elastic moduli from 80-100 MPa to 130-150 MPa. Tracking highlighted that the aligned phase was the mainly strained during mechanical stimulation corresponding to the tendon and muscle area. Myotubes were observed by immunostaining where the C2C12 were seeded, showing their correct trend to muscle fate. Unexpectedly, ALP staining was more ubiquitous in the co-culture than in monoculture (Fig. 2). There was thus no clear evidence in this situation that the microstructures can guide C3H10 differentiation towards bone or tendon lineage.

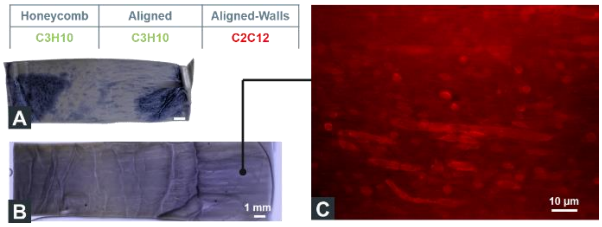

Figure 2: ALP staining after 7 days of dynamic culture in monoculture (A) and coculture (B) conditions. Myotubes immunostained by alpha-actinin (C).

Discussion

This new triphasic microsctuctured scaffold permited to evaluate the capacity of cocultured C2C12 and C3H10 to differentiate into bone, tendon and muscle lineage without any differentiation factor. Our results highlighted that the co-culture attenuated the effect of surface topology on cell differentiation, as previously observed with monoculture. Further investigations are needed to define whether this effect is due to the co-culture medium or by factors released by the cells.

References

1. Garcia Garcia et al, ACS Biomaterials Science and Engineering 4, no 9, 2018
2. Garcia Garcia, et al. Journal of Biomedical Materials Research Part A 109, n° 10, 1881-92, 2021
3. Beldjilali-Labro et al, International Journal of Molecular Sciences, 23(1):260, 2022.
4. Nedjari et al, Materials Letters 142, 180-83, 2015

Acknowledgements

This work was supported by a scholarship from Graduierten Akademie from Leibniz University, by fundings from the French Ministry of Higher Education and Research, by Short-Term Research Grants 2023 from the DAAD, and ANR.