Development of *in vitro* Muscle Tendon Junction Using Aligned Electrospun Polycaprolactone Fibres under Strain

Nodoka Iwasaki[1], Marta Roldo[1], Katerina Karali[2], Gordon Blunn[1]
[1]*School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK,* [2]*School of Mechanical and Design Engineering, University of Portsmouth, Portsmouth, UK*
nodoka.iwasaki@port.ac.uk

Disclosures: No disclosures.

INTRODUCTION: The muscle tendon junction (MTJ) transmits the force generated by muscle to the tendon and ultimately to the bone, and is associated with muscle strains and tears. When a tear occurs at the MTJ, its regeneration is limited due poor vascularisation, and there is no cure for a complete tear [1]. It has been reported that 28% of injuries in the muscle-tendon-bone unit occur at the MTJ [2]. Injuries at the MTJ can significantly affect patients' quality of life by limiting the ability to move, therefore, the development of a tissue engineered MTJ would be beneficial. This project aims to develop a 3D MTJ *in vitro* using electrospun fibres by applying 10% cyclic strain.

METHODS: Polycaprolactone (PCL) fibres were fabricated using electrospinning and a custom-made gap collector and the fibres were characterized by scanning electron microscope (SEM) imaging (EVO MA10, White Plains), fibre diameter and angle distribution analysis (ImageJ software), degradation study and tensile test (Bose ElectroForce 3200, Bose ElectroForce). Human myoblasts and tenocytes (ZenBio) were co-cultured on plastic coverslips with collagen coating (control) or aligned PCL fibres with and without 10% cyclic strain (1 Hz) for 1 hour/day for 7 or 14 days. RT-qPCR was performed to determine the MTJ marker gene such as collagen 22 (Col22) and paxillin (Pax), and immunocytochemistry was performed in order to analyse the expression of MTJ markers and the formation of junction's specific structure, finger-like processes, for the evaluation of the effect of strain and co-culture of myoblasts and tenocytes for the development of an *in vitro* MTJ. All the data were expressed as the mean ± standard deviation (SD), and all experiments were conducted in triplicate. Normal distributions were calculated using Kolmogorov-Smirnov test and either ordinary one-way ANOVA or Two-way ANOVA was performed to calculate the differences ($p < 0.05$) between different sample groups using GraphPad Prism 8.0.2.

RESULTS SECTION: Aligned fibres were successfully fabricated by electrospinning with the use of a gap collector. The aligned fibres promoted cell alignment. It was observed that significantly more cells were aligned ($p < 0.01$) when 10% strain was applied to the fibres (43.9 ± 11.1%) compared to fibres that were not strained (30.2 ± 2.2 %). The average fold expression of co-culture with and without strain relative to 2D co-culture is shown in Figure 1. Col22 and Pax were significantly upregulated ($p < 0.0001$ and $p < 0.01$ respectively) at day 7 and Tal was upregulated at day 14 on the sample with strain. Immunofluorescence images of myoblasts and tenocytes co-culture on PCL fibres subjected strain showed a higher production of Col22 (Figure 2a) and Pax (Figure 2b), especially at day 14, compared to cells co-cultured on plastic or on fibres not subjected to strain. This was confirmed by mean fluorescence intensity analysis (Figure 2c, d). Morphological changes of human myoblasts and tenocytes in repose to culturing conditions were investigated by measuring the aspect ratio and significantly higher aspect ratio ($p < 0.05$) was observed after 14 days for those cells cultured on strained fibres. Mono-cultures of myoblasts and tenocytes were carried out on fibres cyclically strained for 14 days and used as a control in order to demonstrate the effect of co-culturing these cell types on the MTJ formation. Immunocytochemistry images and mean fluorescence intensity analysis showed that the expression of collagen 22 and paxillin was significantly higher ($p < 0.01$) in the co-cultures compared to the mono-cultures obtained in the same dynamic culturing conditions.

DISCUSSION: The effects of strain and co-culture of myoblasts and tenocytes were investigated using RT-qPCR and immunocytochemistry and the results demonstrated that both strain and co-culture of myoblasts and tenocytes are essential to induce upregulation of these MTJ marker genes. The immunofluorescence images illustrated that cells were on the surface of the scaffold and did not migrate into the fibres. In addition, maturation of the MTJ was challenging and the formation of finger-like processes was not observed. Differentiation of cells is another important factor of the MTJ development and adding differentiation factors to this system could induce more mature MTJ formation.

SIGNIFICANCE/CLINICAL RELEVANCE: This study showed that the combination of the strain and co-culture of myoblasts and tenocytes promotes gene expression and protein production seen in the MTJ for the first time.

REFERENCES:

[1] Narayanan and Calve, *Connect Tissue Res*, vol. 62, no. 1, pp. 53–71, 2021, doi: 10.1080/03008207.2020.1814263.
[2] Garrett and Faherty, *Muscle and Tendon Injuries*, 2017, pp. 51–60. doi: 10.1007/978-3-662-54184-5_5.

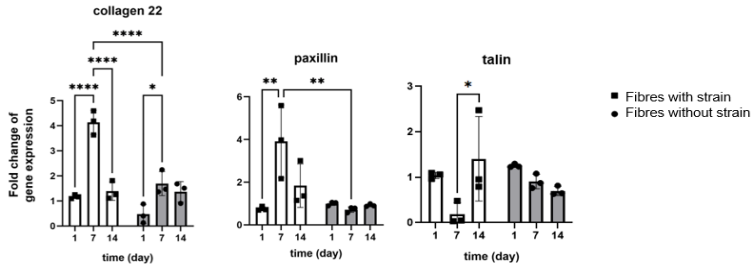

Figure 1. Gene expression of Col22, Pax, and Tal analysed by RT-qPCR. Comparison between co-culture of human myoblasts and tenocytes on aligned PCL fibres with and without strain at day 1, 7, and 14 (n = 3). Data are presented as average fold change relative to 2D co-culture of human myoblasts and tenocytes ± SD. Statistical analysis was carried out between the samples at each angle by two-way ANOVA (* $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$).

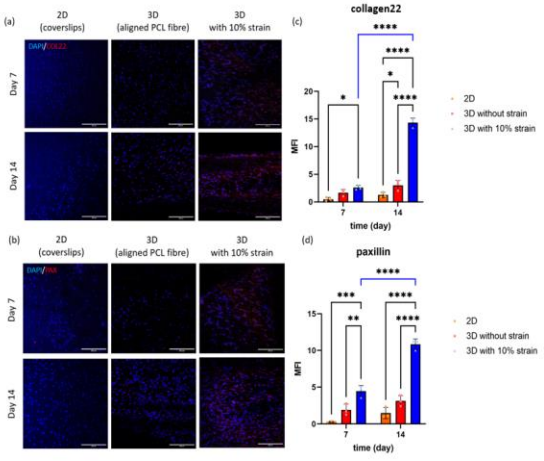

Figure 2. Immunofluorescence images of the MTJ makers at day 7 and 14, and quantitative analysis of the mean intensity fluorescence. Human myoblasts and tenocytes were cultured together in 2D or 3D with/without strain. Representative confocal images of (a) Col22 and (b) Pax at day 7 and 14. Quantitative analysis of mean fluorescence intensity (MFI) of (c) Col22 and (d) Pax using the confocal images. Data are presented as average ± SD (n = 3). Statistical analysis was carried out between the samples and time points by two-way ANOVA (* $p < 0.05$, and **** $p < 0.0001$). Scale bars are 250 μm.