

Tenocyte alignment is dependant upon cell density and tensional loading

[T.Kamarul](#)¹, [M.M.Roebuck](#)², [R.L.Williams](#)³, [S.P.Frostick](#)²

¹*Department of Orthopaedic Surgery, University of Malaya, Kuala Lumpur, Malaysia,*
²*Musculoskeletal research group, Division of Surgery,* ³*Department of Clinical Engineering,*
Royal Liverpool University Hospital, Liverpool L69 3GA, GB

INTRODUCTION: Although it has been accepted that tenocyte responds to mechanical stimulation, (following a process known as mechanotransduction) little is known of its physical response when tensional force is applied through them. Although there have been some reports of cell behaviour in response to tensional loading, these studies have not taken into account *In Vivo* conditions including cell to cell contact, which is major influencing factor. Our study aims to prove that cell contact and different applied strains affect cell behaviour.

METHODS: Primary cell cultures were performed using remnants of biceps tendon tissues from patients undergoing shoulder reconstruction. Cells were passaged to P2 before being seeded onto our custom made collagen type I coated silicone flasks. Different seeding densities were cultured in low-glucose DMEM with 10% foetal calf serum (FCS). Cells were left to grow in these flasks for 4 days prior to loading. These flasks were placed on our custom made motorised tensional loading jig within our culture incubator. While one end is fixed in a static clamp, the flasks are subjected to cyclic unidirectional loading by the motor. Loading strains of approximately 3% and 6% at 1 Hz cyclical loading patterns were applied. Observations were made with photographs of cultured cells taken at specific placement points within the flasks at 1, 3, 6, 24 and 48 hours. Image analyses were performed accordingly.

RESULTS: It was observed that at 6% strain, cells at higher densities showed different alignment patterns than that of lower cell densities (where there are lower or absent cell to cell contacts) favouring an alignment pattern in parallel with the direction of pull. These changes can be seen as early as 6 hours from the start of loading. In contrast, cells which had minimal contact with other surrounding cells appear to be aligned perpendicular to the direction of pull. However, at 3% strain (or less), cells at higher densities appear to align

more perpendicular to the direction of loading. At similar strain levels, cells which are not in contact with each other appear to have little or no response to loading. In addition, they appear to have more rounded morphology at the end of 24 hours.

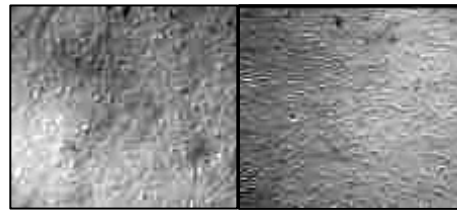


Fig. 1: Pictures taken prior to loading at 6% strain (left) as compared to pictures taken at the end of 24 hours (right) of cells at high densities. Note that the cells are aligned towards the direction of loading

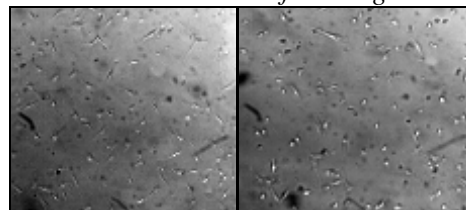


Fig.2: Pictures taken prior to loading at <3% strain (left) as compared to pictures taken at the end of 24 hours (right) of cells at low densities. Note the loss of normal tenocyte morphology.

DISCUSSION & CONCLUSIONS: Our experiments demonstrate that cell seeding density and level of strain are important factors to consider when conducting experiments simulating *In Vivo* conditions. In comparing our experimental model against histological pictures of whole tendon sections, similar cell morphologies were found. Thus this experimental model replicates *In Vivo* conditions.

REFERENCES: ¹M.Eastwood, D.A. McGrouther, R. A. Brown (1998) *Proc Instn Mech Engrs* 212(H):85-92 ² C. Neidlinger-Wilke et al (2001) *J Orth Res* 19:286-293 ³ W.A. Loesberg et al (2005) *J Biomed Mater Res A*. 1;75(3):723-32