

'External loading determines specific ECM genes regulation'

D.Karamichos, J.Skinner R.Brown and V.Mudera

TREC (Tissue Repair and Engineering Centre), Institute of Orthopaedics, University College London, UK

INTRODUCTION: Bio artificial matrices embedded with cells are simulated in bioreactors to facilitate ECM production. As cells attach, they develop forces, which are dependent on cell type and matrix stiffness. External forces (i.e strain), however, are critical for tissue homeostasis and elicit specific cellular responses, such as gene expression³ and protein production. Collagen Type I is a widely used scaffold in Tissue engineering. The aim of this study was to study the mechanical and molecular responses, of different cell types to increasing collagen substrate stiffness².

METHODS: Cultured Human Bone Marrow Stem Cells (hBMSC) and Human Dermal Fibroblasts (HDF) were embedded in collagen constructs in 10% and 20% Fetal Calf Serum(FCS). Constructs were then pre-strained (0%, 5% and 10%) to increase matrix stiffness. Contraction forces generated by cells were quantified for 24 hours on the tensional Culture Force Monitor¹ after allowing for visco-elastic relaxation of collagen. ECM regulatory molecular genes were quantified using real time RT-PCR.

RESULTS: hBMSCs showed FCS dependent contraction forces, in contrast to HDFs which showed matrix stiffness dependent. 10% FCS concentration significantly ($p=0.05$) reduced peak contraction as pre strain was increased in both hBMSC's (Figure 1) and HDF's ($0% > 5% > 10%$). HDF's had significantly increased force generation (twice as much) to increasing serum concentration (i.e 10% to 20%) however contraction was still significantly reduced with increasing substrate stiffness in 20% FCS. In contrast, hBMSC's at 20% FCS generated similar peak force contraction at 24h irrespective of pre strain ($0%=5%=10%$, Figure 2). ECM regulatory genes MMP2 and MMP9 showed up regulation at 5% pre strain, but a 50% down regulation when pre strain was increased to 10%. COL3 was down regulated at 5% and up regulated at 10% pre-strain.

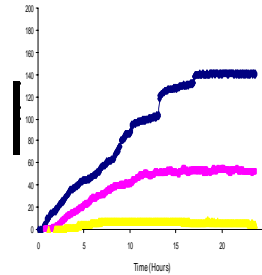


Figure 1. hBMSC's contraction force response to 0%-10% pre-strain (10% FCS)

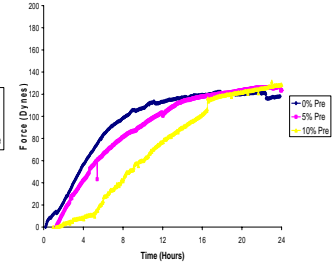


Figure 2. hBMSC's contraction force response to 0%-10% pre-strain (20% FCS)

DISCUSSION & CONCLUSIONS: We have shown significantly differential mechanical and molecular response of hBMSC's to increased substrate stiffness and increased FCS (20%) stimulation compared to HDF's. For predictable cellular responses, mechanical stimulation of cells will have to be tailored to take into account the increasing stiffness of the matrix as ECM is deposited, as well as cell phenotype and serum concentration.

REFERENCES:

¹M.Eastwood, V.C.Mudera, D.A.McGrouther, R.A.Brown (1998) Effect of precise mechanical loading on fibroblast populated collagen lattices: Morphological changes. *Cell motility and the cytoskeleton* **40**:13-21

²Karamichos D, Mudera VC, Brown RA. 2006. Complex dependence of substrate stiffness and serum concentration on cell-force generation. *J Biomedical Materials Research: Part A* Accepted for publication

³V.C.Mudera, R.Pleass, M.Eastwood, R.Tarnuzzer, G.Schultz, P.Khaw, D.A.McGrouther, R.A.Brown (2000) Molecular responses of human dermal fibroblasts to dual cues: Contact guidance and mechanical load. *Cell motility and the cytoskeleton* **45**:1-9

ACKNOWLEDGEMENTS: This study is funded by a Engineering and Physical Sciences Research Council Grant.