THE ROLE OF ADHESION JUNCTIONS AND FLUID FLOW IN THE STIMULATION OF OSTEOGENESIS

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Introduction

Early bone cells (MC3T3-E1s) are known to be sensitive to fluid shear stress (FSS) stimulus and regulate their metabolic and adaptive functions in response [Bakker 2001] but the mechanism through which they do this remains unclear. Adhesion junctions (AJs) form an extracellular link between the cyto-skeletons of adjacent bone cells and it has been shown that FSS stimulates an AJ osteogenic signalling response [Norvell 2004]. The objectives of this study are to investigate the role of AJs in the mechanotransduction of FSS into an cellular osteogenic response and to develop a fluid-structure interaction (FSI) model to examine and predict the role of AJs in osteogenesis

Methods

MC3T3-E1s were seeded on collagen type-I coated glass slides and grown to confluency in MC3T3-EI expansion media. Samples were divided into 3 treatment groups; recombinant human Endostatin (Peprotech) which degrades the AJ signalling molecule β-catenin, EGTA (Sigma) which chelates extracellular Ca^{2+} and a control. After treatment, FSS of 1 Pa was applied for 1 hr in a parallel plate bioreactor [Lane, 2012]. Cellular response is analysed using immunofluorescent staining, ALP, PGE_2 and NO assays and RT-PCR. Using Ansys software a FSI model was developed to compute the flow induced strain distribution in two MC3T3-EIs connected by a single AJ. Cells were oriented at 0^o and 90^o to the flow direction to assess the influence of AJ location on computed strain concentrations. The cell nucleus and cytoplasm were modelled as elastic materials.

Results

A significant decrease in AJs in EGTA treated samples was observed (Fig. 1A). FSS induced realignment of the cytoskeleton and stress fibre formation are most pronounced in EGTA FSS samples. ALP results were analysed using two-way ANOVA, followed by pairwise multiple comparison procedures (Tukey test). A significant ($p \leq 0.05$) decrease in ALP activity was observed for EGTA treated samples. A significant increase in ALP activity was observed for control FSS samples, but no significant difference was found between EGTA static and EGTA FSS samples. FSI simulations (Figure 1B) reveal that a significant strain concentration occurs in the region of the AJ when cells are oriented at 90^o to the flow direction, in sharp contrast to computed results when cells are oriented at 0^o.

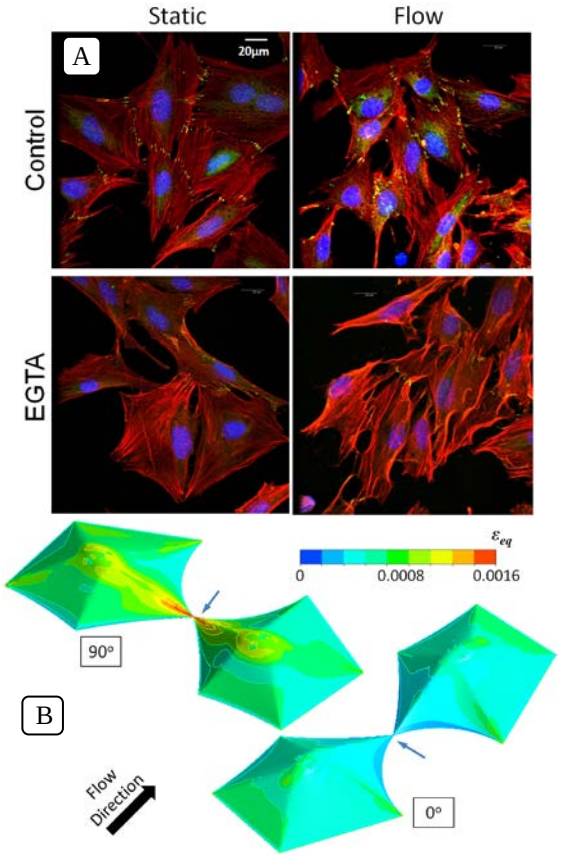

Figure 1: A) MC3T3 Immunofluorescent staining of N-cadherin AJs (green), cytoskeleton (red) and the nucleus (blue). B) Contour plot of equivalent strain on idealised MC3T3-EI models joined by AJs (blue arrows).

Discussion

ALP results indicate that AJs could be significantly involved in MC3T3 osteogenesis, while FSI results indicate that the mechanical stimulus received by an AJ is highly dependent on location and orientation to the flow field. Simulation of cell morphologies observed in-vitro will further elucidate the role of fluid flow and AJs in cell remodelling.

References

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