Bioreactor culture of tissue engineered bone increases matrix production by subjecting the growing tissue to mechanical forces and/or fluid flow. For this to occur bone cells must be able to sense mechanical load in order to impart effective changes in the tissue architecture. In bone the movement of pericellular fluid, between the cell membrane and the wall of the tissue, induced by dynamic mechanical loading, has been proposed to be sensed by osteocytes via the cell coat (glycocalyx) [1]. But it is unknown if this mechanism also operates in the matrix producing bone cells that would receive the flow stimulus during bioreactor conditioning. The aim of the current work was to elucidate some of the signalling mechanisms by which fluid flow increases long term collagen production in particular examining the contribution of the hyaluronan (HA) glycocalyx as a possible mediator of mechanotransduction.

Materials and Methods
Using a parallel plate flow chamber (µslide VI, Ibidi, Munich Germany) we investigated the effects of enzymatic removal of HA or antibody blocking of the HA binding protein CD44 on flow induced collagen production and NF-κB activation in MLO-A5 osteoid osteocytes [2]. Static culture was also utilised to investigate pathways linking Ca\(^{2+}\) responses, NF-κB activation and long term collagen production.

Results
Two hours of fluid flow at 0.8Pa shear stress induced nuclear translocation of the NF-κB subunit, p65 in 65% of the cell population (Fig 1). Two bouts of two hrs of flow within 10 days of culture significantly increased collagen production. Blocking of CD44 and removal of the HA coat and, had no effect on the translocation of p65 but eliminated the fluid flow induced increase in collagen production (Fig 2). In statically cultured MLO-A5 cells addition of ionomycin, to stimulate a Ca\(^{2+}\) response, appeared to reduce the lipopolysaccaride (LPS) stimulated activation of p65.

Discussion and Conclusions
We used a 2D parallel plate flow chamber system to apply a defined flow profile to MLO-A5 cells to determine the mechanisms of the mechanical response. hyaluronan and CD44 appear to play a role in transducing long term flow signals that modulate collagen production but do not effect short term flow-induced activation of NF-κB. It is likely that multiple signalling events are initiated upon the commencement of fluid flow in tissue engineered bone and it is has yet to be clarified which of these ultimately contribute to the matrix forming response.

References

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Disclosures
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