were obtained when each zone of AC was analysed separately and also when data for MCP and MTP joints was analysed individually.

Conclusions: The apparent linear increase in chondrocyte apoptosis at early stages of AC degradation (‘modified’ Mankin grades 0-3) suggests that this process may play a significant role in the initiation of cartilage damage. This data therefore supports the notion that cell death by apoptosis may be an important aetiopathogenic mechanism in OA.

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DYNAMIC COMPRESSION COUNTERACTS FIBRONECTIN FRAGMENT INDUCED UPREGULATION OF NITRIC OXIDE

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Purpose: The importance of nitric oxide (*NO) as a key mediator in the pathophysiology of osteoarthritis (OA) has been well documented. The underlying mechanism involves the induction of *NO and prostaglandin E₂ (PGE₂) by pro-inflammatory cytokines, that lead to the degradation of the extracellular matrix constituents. Fibronectin is a component of the extracellular matrix. In OA, fibronectin is degraded into small fragments and these degradation products could play a major role in cartilage breakdown. Recent studies have shown that fibronectin fragments (Fn-F) enhance the release of *NO and/or matrix metalloproteinase (MMP) enzymes. However, these studies focused primarily on the pathways in the absence of mechanical stimuli. We have previously shown that dynamic compression counteracts *NO and PGE₂ release in the presence of IL-1β. The current study examines the biochemical changes observed by chondrocyte/agarose constructs subjected to dynamic compression in the presence of Fn-F.

Methods: Initial dose response studies were conducted under free-swelling conditions in order to determine the appropriate concentration of Fn-F for the mechanical loading studies. Chondrocyte/agarose constructs were cultured for 48 hours in radio-labelled media containing 0, 0.01, 0.1, 1 or 10 μg ml⁻¹ of the heparin binding 40 kDa Fn-F, +/- 1 mM L-NIO (NOS inhibitor). For the mechanical loading studies, constructs were subjected to 15% dynamic compressive strain at 1 Hz frequency for 48 hours in media supplemented with 0 or 10 μg ml⁻¹ Fn-F, +/- 1 mM 1400 W (iNOS inhibitor). Nitrite and PGE₂ release, [³H]-thymidine and 35SO₄ incorporation were measured using well established biochemical assays.

Results: Fn-F at concentrations of 0.1, 1 and 10 μg ml⁻¹ increased nitrite release when compared to control constructs (all p<0.001). L-NIO downregulated nitrite release when constructs were cultured with 10 μg ml⁻¹ Fn-F (p<0.05). By contrast, Fn-F did not significantly influence PGE₂ release in the presence and absence of L-NIO. [³H]-thymidine incorporation was downregulated with 1 and 10 μg.ml⁻¹ Fn-F when compared to control constructs (p<0.05 and p<0.001). 10 μg.ml⁻¹ Fn-F inhibited 35SO₄ incorporation when compared to control (p<0.001) and this response was reversed by L-NIO (p<0.01). In unstrained constructs, 10 μg.ml⁻¹ Fn-F upregulated nitrite release when compared to control constructs (p<0.001). Dynamic compression inhibited nitrite release in the absence and presence of Fn-F (both p<0.001) and this effect was abolished with 1400 W. Dynamic compression in the presence and absence of Fn-F did not significantly influence PGE₂ release (Fig. 2B). In unstrained constructs, Fn-F and 1400 W increased PGE₂ release when compared to control constructs (p<0.01). This effect was not influenced by dynamic compression. In the absence of Fn-F, dynamic compression upregulated [³H]-thymidine (p<0.001) and 35SO₄ incorporation (p<0.01). The upregulation of [³H]-thymidine and 35SO₄ incorporation by dynamic compression was abolished with Fn-F and enhanced with 1400 W (p<0.001 and p<0.01, respectively).

Conclusions: The current study demonstrates that Fn-F stimulates *NO release and this effect could be abolished by mechanical load. PGE₂ levels were not influenced by the presence of Fn-F, suggesting alternative pathways which could activate IL-1β and/or MMP expression. Thus, a suppression of *NO is important in mediating the upregulation of cell proliferation and proteoglycan synthesis in response to dynamic compression. Further characterization of the mechanotransduction pathway in the presence of inflammatory mediators may identify pharmacological targets for the treatment of OA.