DEVELOPMENT OF A NOVEL CULTURE SYSTEM TO STUDY THE INFLUENCE OF CYCLIC TENSILE STRAIN ON THE MACROPHAGE RESPONSE TO PARTICULATE WEAR DEBRIS IN VITRO

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Introduction: The biological response to implant-derived wear debris (e.g., UHMWPE, PMMA) is now known to play a major role in the early failure of total joint replacements by aseptic loosening. Histological studies have detected macrophages and giant cells in association with particulate debris, along with a range of osteolytic mediators, in the tissues surrounding loosened implants. In addition, human macrophages have been shown to secrete these factors in response to small polyethylene particles in vitro. However, micromotion and mechanical strain, within the interfacial soft tissues, may also contribute to this process and the relative contributions of wear debris and cyclic strain to loosening in vivo, as yet remain unclear. Whilst a previous study demonstrated that cyclic compressive strain was able to act synergistically to up-regulate the production of TNFα by particle stimulated macrophages, little is known about the response to cyclic tensile strain. We have therefore designed a device (‘Ten-Cell’) to apply micromechanical forces to 3-D culture systems in vitro. Using TNFα, a key mediator of osteolysis, as the marker of macrophage activation, this study investigated the role of cyclic tensile strain on the osteolytic potential of polyethylene particle stimulated mononuclear phagocytes cultured within a soft tissue equivalent model.

Materials and Methods: ‘Ten-Cell’ is a servo-controlled multi-station cyclic strain device, comprising eight static control wells and eight mobile test wells. Uniaxial displacements of 0 – 4mm can be applied to samples up to 10mm in length, with a sinusoidal waveform (resolution = 10µm). The device is housed within a chamber to maintain high humidity, physiological temperature and a buffering atmosphere.

For this study, mononuclear cells were isolated from peripheral blood by density centrifugation and the proportion of mononuclear phagocytes (MP) determined using a latex bead ingestion assay. Cells (10^4 MP/ml) were resuspended in 2% (w/v) Matrigel™ (Beckton Dickinson,), with or without polyethylene (PE) particles (fractionated Ceridust® 3615®; mean size: 0.21±0.07µm; Hoechst, Germany) at 30µm/cell. Gels plus particles and/or MP, supported on silicon sheets for clamping into the device, were then transferred to the test wells and either cultured statically or subjected to 20% cyclic tensile strain (frequency of 1Hz) in the ‘Ten-Cell’ for 16 hours. Cell viability (ATP-lite™ luminescence assay; expressed as ‘Counts per second’) and TNFα secretion (ELISA; expressed as the mean ‘Specific Activity’; ng/ml cytokine/C.P.S. ÷ 10^6) were then quantified. Positive (cells + LPS) and negative (cells + matrix) controls were included. Results were then analysed by one-way ANOVA and calculation of the minimum significant difference (T-method).

Results: Previous studies demonstrated that a particle volume to cell number ratio of 30µm^3 of debris per macrophage was sub-optimal for cell activation, resulting in little or no TNFα response in vitro. However, particle volumes greater than this, induced the secretion of significantly elevated levels of cytokine compared with unstimulated controls. As the aim of this study was to determine any synergistic effect of cyclic strain on the particle response, macrophages were stimulated with polyethylene particles at the sub-stimulatory concentration of 30µm^3 per cell. A following exposure to 20% cyclic tensile strain, cell viability was observed to be reduced compared with unstrained, static controls (Fig. 1). These results were significant at the p<0.05 level. No increase in TNFα production was observed when cells were subjected to either cyclic tensile strain or particle stimulation alone, compared with the cells only negative control. However, significantly elevated levels of TNFα were measured in culture supernatants from cells that had been subjected to particle stimulation in conjunction with 20% cyclic tensile strain (p<0.01), compared with the cells only, cells plus cyclic strain and cells plus particle stimulation only treatment groups (Figure 2).

Discussion: Using ‘Ten-Cell’, we have now begun to study the biological effects of cyclic tensile strain and cyclic tensile strain plus polyethylene wear debris on the production of TNFα by macrophages in vitro. In this study, these two factors have been shown to be able to act synergistically, to up-regulate the production of TNFα by human mononuclear phagocytes co-cultured in vitro with low, sub-stimulatory concentrations of polyethylene particles. However, further work is now required to establish whether cyclic strain is acting directly upon TNFα transcription or whether the observed effects result from e.g., enhanced phagocytic activity or delivery of particles to the cells. Future studies will address this question. However, this study has clearly demonstrated that at low particle load, cyclic tensile strain may contribute significantly to macrophage activation and osteolytic cytokine production in vitro and, ultimately, in vivo.

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References

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