Modulating Macrophage Response To Wear Particles To Mitigate Peri-Implant Osteolysis

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Background: Total joint replacements have been widely used to return mobility to patients suffering from arthritis; however a considerable number of these fail due to periprosthetic osteolysis generally believed to be caused by wear debris generated during mechanical loading of the joints[1]. Ultra high molecular weight polyethylene (UHMWPE) components are used in majority of artificial joints in US and hence submicron size UHMWPE wear particles are the most abundant and reactive type of wear particles retrieved from the periprosthetic tissue during revision surgeries[2]. Upon phagocytosing wear particles, macrophages are activated and release cytokines such as IL-1beta, IL-6 and TNF-alpha[3]. These cytokines have been shown to activate osteoclasts and cause bone resorption around implants[1]. Wear debris particles generated in the body are coated with adhesive proteins which facilitate macrophage adhesion and thus activation. Integrins, such as Mac-1 mediates macrophage adhesion to adsorbed proteins. In this work we aim to identify receptors responsible for macrophage adhesion to and phagocytosis of UHMWPE wear debris. Identification of the primary mediator in the osteolytic response will enable targeting of integrin receptors for anti adhesion therapies for peri-implant osteolysis.

Methods: Macrophages matured from bone marrow stem cells harvested from C57BL6 mice and incubated with Macrophage Colony Stimulating Factor (MCSF) for 5 days or RAW 264.7 murine macrophage cells were used to study macrophage phagocytosis. UHMWPE particles coated with serum were incubated with either macrophages or RAW 264.7 cells overnight using the inverted cell culture system**(Fig 1)**. The number of UHMWPE particles phagocytosed, were visualised using Oil Red O staining**(Fig 2)**. In order to determine the number of RAW 264.7 macrophages phagocytosing particles, cells were fixed permeabalized and immunofluorescently stained for VATPase subunit E a mature phagosome marker**(Fig 3)**. Supernatant was collected for cytokine analysis for TNF-α and IL-1β using ELISA.

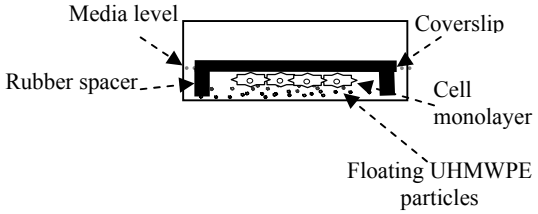

Figure 1. Inverted macrophage culture system: detail of a single well. UHMWPE, due to its low density compared to water, float to the surface. In order to ensure contact between macrophages and floating particles, macrophages are using an inverted configuration.

Results and Discussion: This data demonstrates the feasibility of the inverted culture system to examine macrophage phagocytosis of UHMWPE particles. This system has an advantage over other systems such as UHMWPE particles fixed onto the bottom of the plate using collagen or culture medium as it allows free interaction between the macrophages and particles similar to that within the body. Mac-1 receptor plays a primary role in mediating macrophage molecular recognition and response to wear debris particles. In order to quantify the role of Mac-1 in macrophage activation, macrophages from Mac-1 knockout mice will be isolated and the phagocytosis of UHMWPE particles will be studied. The UHMWPE particles will be coated with different Mac-1 ligands such as fibronectin, vitronectin, BSA in order to compare its binding to different proteins. To quantitate the phagocytosis and subsequent macrophage activation, Oil Red O staining, phagosome staining , cytokine analysis and integrin binding using a biochemical cross-linking/extraction method[4]will be performed. The results will be compared with a wild type control to isolate the effect of Mac-1. Identification of the key mediators of the osteolytic response will enable design of therapeutics such as anti-adhesion therapy using integrin receptor targeting.

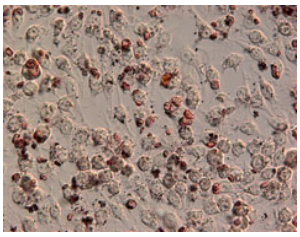

Figure 2. Oil Red O staining of PE microparticles visualized by DIC microscopy. This validates efficacy of inverted cell culture system as PE microparticles are shown phagocytosed by macrophages.

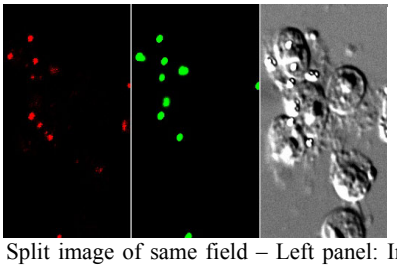

Figure 3. Macrophage phagocytosis of fluorescent microparticles, is demonstrated by co-localization of mature phagosomes and fluorescent microparticles using the V-ATPase subunit E immunofluorescence staining Split image of same field – Left panel: Immunofluorescence stain of V-ATPase subunit E, a mature phagosome marker. Middle panel: FITC-labeled 1μm PS beads incubated with macrophages. Right panel: DIC image of macrophages with beads.

References
[1] Bauer, T. W. & Schils, J. The pathology of total joint arthroplasty.II. Mechanisms of implant failure. *Skeletal Radiol.* **28**, 483-497 (1999).
[2] Mabrey, J. D. *et al.* Standardized analysis of UHMWPE wear particles from failed total joint arthroplasties. *J. Biomed. Mater. Res.* **63**, 475-483 (2002)
[3] Ingham, E. & Fisher, J. The role of macrophages in osteolysis of total joint replacement. *Biomaterials* **26**, 1271-1286 (2005)
[4] Keselowsky, B. G. & Garcia, A. J. Quantitative methods for analysis of integrin binding and focal adhesion formation on biomaterial surfaces. *Biomaterials* **26**, 413-418 (2005).