Cytokine Expression from Monocytes/Macrophages Response to Ti-particles and Discs

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Introduction: Monocytes/macrophages are the sentinel cells that direct the inflammation and wound healing responses to biomaterial implants via secretion cytokines and growth factors [1]. The temporal release profiles of these cytokines and growth factors in response to a biomaterial thus reflect the material's potential biocompatibility [2]. In general, it is thought that monocytes/macrophages do not respond to the presence of medical-grade materials unless they degrade or are in particulate form. This study examines whether monocytes produce a significant cytokine expression profile in response to Ti in both bulk and particulate forms.

Methods: Human THP-1 monocytes were added to a 24 well plate at a density of 10^5 cells/mL. The cells then received one of three treatments with titanium: 1) addition of Ti particles at 1 mg/mL, 2) addition of Ti particles at 0.1 mg/mL, or 3) addition of a titanium disc. LPS (Lipopolysaccharide) at a concentration of 1 μg/ml was added into designated wells as a stimulus. The cell culture was interrupted at 1, 6, 24, 48, and 72 hrs. Cell viability was determined based on exclusion of Trypan Blue Staining. Aliquots of cell culture supernatant were sampled at different time points and assayed for inflammatory and wound healing cytokines using multiplexed immuno-enzymatic assays that operate in a sandwich fluoro-immunoassay mode.

Results/Discussion: Phase contrast images of cells adjacent to the culture plates either with (Figure 1 (b) and (d)) or without Ti particles (Figure 1 (a) and (c)) were collected 72 h after seeding without (Figure 1 (a) and (b)) or with (Figure 1 (c) and (d)) 1 μg/ml LPS stimulation. Cells arrowed in Figure 1 (c) indicate a flattened, differentiated morphology due to LPS stimulation. Unstimulated THP-1 monocytes on culture plates maintained a rounded, undifferentiated morphology as expected for a hydrophilic surface (Figure 1 (a) and (b)).

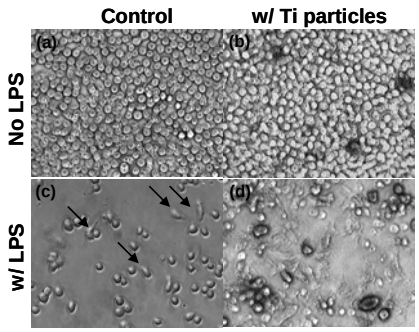

Figure 1. Effect of Ti particles on THP-1 monocyte differentiation

With the addition of Ti particles, many monocytes displayed a balloon-like appearance resulting from a marked increase in cytoplasm. Furthermore, phagocytosed particles were found in a significant fraction of cells (Figure 1 (a) and (d)). In all cases, cell viability at 72 h was essentially 100%. Of the 10 cytokines assayed, cytokines were barely detected in the supernatant of cells cultured on the control sample. However, cells subjected to any combination of LPS or titanium treatment produced substantial cytokine expression from monocytes. Wells with titanium particles and discs caused a substantial increase of inflammatory cytokine expression (TNF-α, IL-6 and MIP-1α) compared to the control wells regardless of the presence of LPS stimulation. Despite the increased expression of inflammatory cytokines, there was no significant correlation between wound healing cytokine expression and treatment type.

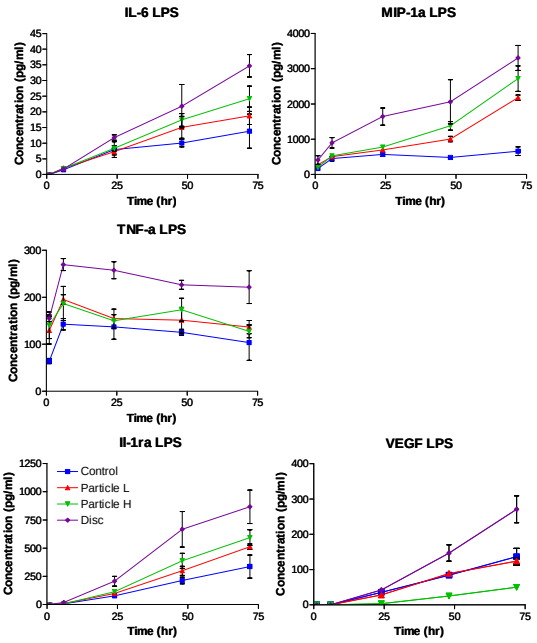

Figure 2. Cytokine Expression of Human THP-1 monocytes cultured on Ti particles and discs with LPS

Conclusions: The cytokine expression profile from Ti discs and particles shows that monocytes/macrophages do not only respond to phagocytable materials, but also respond to non-phagocytable materials. The surprising observation of this study was that inflammatory cytokine expression from the Ti disc was higher than particles. Note that both Ti particles and discs were endotoxin free as examined by Limulus Amebocyte Lysate (LAL) test.

References:

1. Thomson AW et. al, The cytokine handbook, 4 ed. 2003, San Diego: Academicpress.
2. Li YW et. al, Protein array method for assessing in vitro biomaterial-induced cytokine expression. Biomaterials. 2005; 26(10): 1081-1085.