doi: 10.1017/S143192760909850X
Microsc Microanal 15(Suppl 2), 2009
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Apatone® Treatment Inhibits the Inflammatory Response in Human Synovial Fibroblasts Following Metal Particulate Exposure by Reducing NF-κB Levels

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Previous studies have demonstrated that wear particles stimulate host inflammatory and osteolytic responses following total joint replacement. Amelioration of the inflammatory response following metal wear debris exposure could lead to greater stability of the joint tissue interface and extend the life-span of the prosthetic implant [1]. In the current study, energy dispersive spectroscopy (EDS) and X-ray photoelectron spectroscopy (XPS) were employed to respectively identify the bulk and surface metal composition of two ASTM F75 cobalt–chromium–molybdenum (CoCrMo) powders known to produce mild-to-moderate cytotoxic effects. Confluent synovial fibroblasts cultured in T25 flasks ($\approx 3x10^6$ cells) were exposed to 0.004g and 0.04g mass dosages of these same particulate powders to evaluate cellular toxicity. To evaluate the cellular activity of synovial fibroblasts following metal powder exposure, an aliquot of the cultures cells ($\approx 4x10^5$ cells) were stained with Acridine orange, by standard protocol, to evaluate the loss of lysosomal integrity during cell death.

To determine the effectiveness of Apatone® (IC-Medtech, El Cajon, CA), a drug currently under phase-II clinical trials as a chemo-adjuvant and shown to modulate the inflammatory response via down-regulation of nuclear factor kappa B (NF-κB) [2], additional synovial exposure studies were performed. Here synovial fibroblasts were treated with Apatone prior to, or following, their exposure to the F75 CoCrMo particulates previously described. We hypothesize that Apatone conditioning of synovial fibroblasts will reduce, or retard, the cellular cytotoxicity and bioreactivity customarily seen following metal particulate exposure by reducing the oxidative cellular stress and diminishing cellular levels of NF-κB.

While the bulk composition of the metal powders was essentially identical to that of the ASTM stsandard, the XPS scans determined that significant metallic surface segregation was apparent for both (see Table 1). An ANOVA indicated that the type of metal powder used significantly affected fibroblast viability ($p < 0.0001$) with a 0.004 g dosage of CoCrMo-I powder reducing cell viability by 11%, while the same exposure dosage of CoCrMo-II powder reduced cell viability by 86%. At a dose of 0.04g the CoCrMo-I powder decreased viability by 30% while the CoCrMo-II powder decreased viability by 97%. Acridine orange staining demonstrated that fibroblasts tolerated the CoCrMo-I powder exposure with minimal cellular distress or cell death (Fig.1). However, fibroblasts exposure to CoCrMo-II caused e rapid cellular distress with lysosomal leakage within 15 min (Fig. 1). The time course of the cell death when it did occur suggested that it was apoptotic.

Conditioning of synovial fibroblasts with Apatone only was shown to increase cellular proliferation (trypan blue exclusion) to 104% and 167% of control values at 24hrs and 48hrss, respectively (Fig2). Fibroblast viability remained relatively constant following exposure to the metal only (Fig 2). Apatone conditioning 24hrs prior to 24hrs and 48hrs metal exposure resulted in viabilities of 106% and 117%, while viability remained constant at 106% and 108% when Apatone treatment followed metal treatment. NF-κB levels (determined using an EZ-Detect NFκB p65 Transcription Assay Kit) rose to 120% of control levels by 24hrs and then decreased to 79% of control levels by

https://doi.org/10.1017/S143192760909850X Published online by Cambridge University Press

48hrs for the Apatone only. For the metal only exposure the NF-κB levels increased to 149% of control levels by 24hrs and 94% by 48hrs. When compared to the control, Apatone pretreatment led to metal induce NF-κB levels of 49% and 58%. Apatone post-treatment led to metal induced NF-κB values of 109% and 31% (Fig. 2). These results suggest Apatone conditioning may manage cellular cytotoxicity and bioreactivity by reducing NF-κB activation.

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Table 1

	CoCrMo-I EDS[1]	CoCrMo-I XPS[2]	CoCrMo_II EDS[1]	CoCrMo_II XPS[2]	F75CoCrMo ASTM Standard
Cobalt	62%	30%	62%	69%	57.4-65%
Chromium	34%	30%	32%	28%	27-30%
Molybdenum	3%	5%	4%	3%	5-7%
Silicon	1%	27%	2%	0%	1% max
Manganese	0%	8%	0%	0%	1% max

[1]experimental uncertainty 2%; [2]experimental uncertainty <5%

Figure 1. Acridine Orange Staining

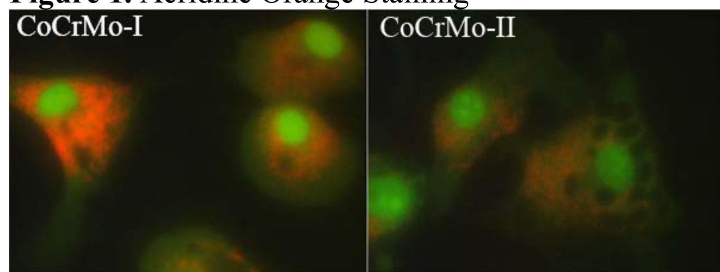
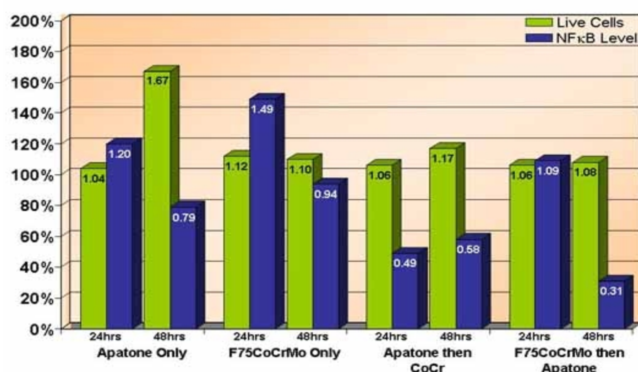


Figure 2. Effect of Apatone on Cell Viability and NFκB Levels of Synovial Fibroblasts



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