Institute of Medical & Biological Engineering

Biological effects of prosthetic cobalt-chromium nanoparticles on cells of the dural membrane

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INTRODUCTION

The increased popularity and success of metal-on-metal (MOM) hip implants has resulted in the emergence of similar MOM prostheses for use in total disc replacement (TDR). However, wear debris generated from such metal prostheses can lead to adverse tissue reactions as reported in recent studies [1, 2]. Consequently, adequate evaluation of the cellular responses to metal wear debris generated by artificial intervertebral discs is warranted.

The aim of this study was to investigate the biological effects of clinically relevant metal nanoparticles on cells isolated from the dura mater, a tissue in close proximity to spinal implants.

METHODS

Particles were generated in a 6 station pin-on-plate wear rig. The bearing combination studied comprised medical grade wrought cobalt-chrome (CoCr) alloy ASTM F1537 pins and plates. Wear particles were then sterilised and were characterized using FEGSEM and Image Pro-plus [3].

Porcine dural fibroblasts and dural epithelial cells were isolated from the dura mater.

The cells were cultured with CoCr nanoparticles at particle volumes ranging from 0.06 μm^3 to 121 μm^3 per cell.

The resulting biological effects were evaluated using a range of cell-based assays.

Cell viability was assessed using the ATP-lite™ assay at 24 hour intervals over 4 days.

Production of the pro-inflammatory cytokines IL-8 and IL-6 in the culture supernatants was determined by enzyme-linked immunosorbent assay (ELISA).

Oxidative stress was analysed using 5 - (and - 6) - carboxy - 2', 7'- dichlorodihydrofluorescein diacetate (carboxy - H_2DCFDA) as a fluorescent probe after a 24 hour exposure of the cells to CoCr nanoparticles.

RESULTS

Particles were uniformly round, oval or irregular in shape with a mode size of 40-49 nm (Fig. 1).

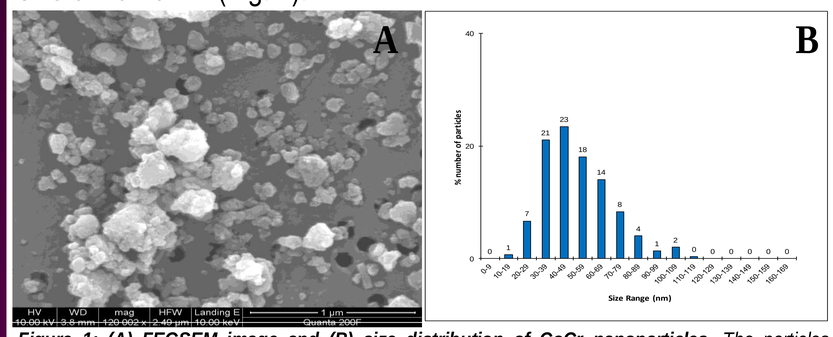

Figure 1: (A) FEGSEM image and (B) size distribution of CoCr nanoparticles. *The particles visualised were found to be clinically relevant with a round, oval or irregular morphology and a mode size of 40-49nm.*

Differential effects of the CoCr nanoparticles were observed on the dural fibroblasts and epithelial cells. The particles significantly reduced the viability of the epithelial cells ($p<0.05$ ANOVA) in a dose and time-dependent manner but not the fibroblasts when compared to the cell only negative control (Fig. 2).

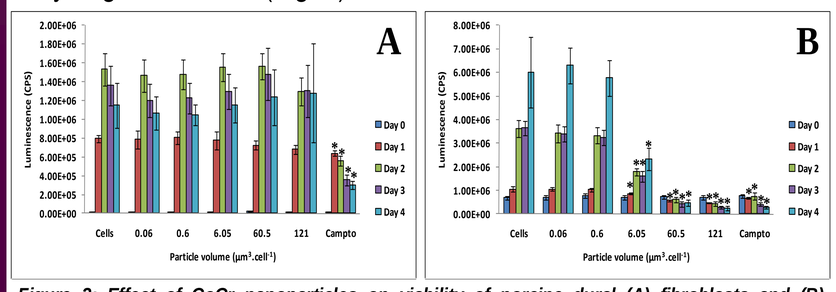

Figure 2: Effect of CoCr nanoparticles on viability of porcine dural (A) fibroblasts and (B) epithelial cells. *Differential effects of the particles were observed on the dural fibroblasts and epithelial cells. The particles significantly reduced the viability of the epithelial cells in a dose-dependent manner but not the fibroblasts when compared to the cell only negative control. Data is expressed as the mean (n=6) ± 95% confidence limits. *p<0.05 ANOVA.*

Both the fibroblasts and epithelial cells were induced to secrete IL-8 when cultured with the particles at doses of either 60.5 or 121 μm^3 per cell or both (Fig. 3).

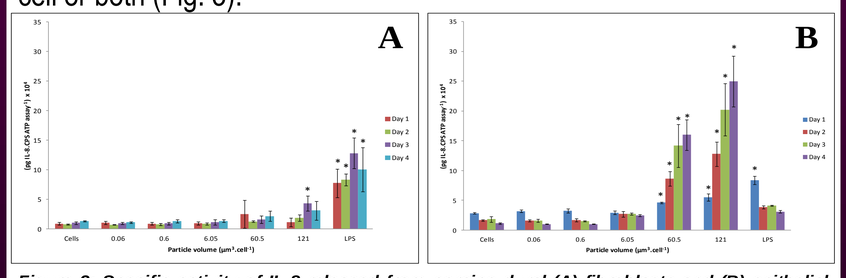

Figure 3: Specific activity of IL-8 released from porcine dural (A) fibroblasts and (B) epithelial cells exposed to CoCr nanoparticles. *Differential effects of the particles were also observed on the production of IL-8 from both cell types. The particles significantly increased the production of IL-8 from the fibroblasts only at the highest dose of 121μm³ per cell at day 3 of culture while the epithelial cells were consistently induced to secrete significantly higher levels of IL-8 at doses of 60.5μm³ and 121μm³ per cell after 1 day of culture with the particles. Data is expressed as the mean (n=3) ± 95% confidence limits. *p<0.05 ANOVA.*

RESULTS

No significant release of IL-6 was observed in both cell types at all doses.

Evaluation of particle-induced oxidative stress revealed the generation of reactive oxygen species in both cell types at a dose of 50 μm^3 per cell after 24 hour exposure (Fig. 4).

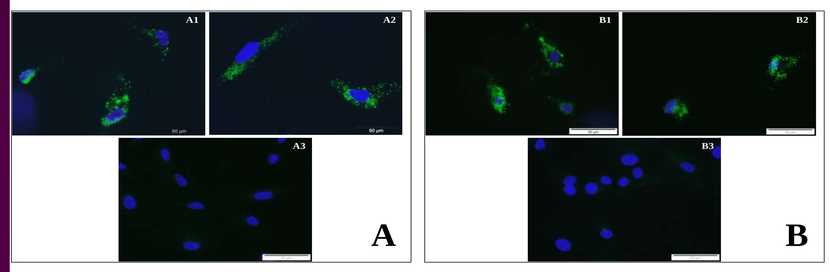

Figure 4: Effect of CoCr nanoparticles on intra-cellular oxidative stress in porcine dural (A) fibroblasts and (B) epithelial cells. *CoCr nanoparticles were shown to have induced the generation of reactive oxygen species in both cell types irrespective of their biological response to particle-induced toxicity. (A1 & A2) and (B1 & B2) Strong or very strong fluorescence staining observed in cells treated with CoCr nanoparticles at a volume of 50μm³ per cell. (A3 & B3) Relatively weak or no staining observed in the cell only negative control at the same particle dose. Cells were visualised under 400x magnification and exposure of 186ms.*

DISCUSSION

- The survival of the dural fibroblasts at doses cytotoxic to the epithelial cells suggested novel differences in the resistance of the two cell types to metal nanoparticle-induced toxicity.
- The difference in this particle-induced toxicity may be regulated, in part, by a difference in their susceptibility to reactive oxygen species.
- Moreover, secretion of IL-8 by the epithelial cells and fibroblasts upon culture with CoCr nanoparticles indicated the inflammatory potential of the wear particles.
- The results generated from this study contribute to a greater understanding of the potential risks associated with the use of MOM total disc prostheses.

ACKNOWLEDGEMENTS

This work was funded in part by NIH Bioengineering Partnership grant R01 AR052653-01 and through WELMEC, a Centre of Excellence in Medical Engineering funded by the Wellcome Trust and EPSRC, under grant WT 08890812/09/Z.

References

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3 Brown et al., 50th Ann. Meet. ORS, 2004