The Effect of Wear Particles from Hard-on-Hard Total Hip Replacements on the Cell Plasma Membrane

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Introduction

Over 94 000 total hip replacements (THRs) were performed in the U.K from April 2012 to March 2013[1]. Under optimum conditions, cobalt chromium (CoCr) metal-on-metal THRs have low wear rates and some studies report no measurable wear for ceramic-on-ceramic bearings[2,3].

However, larger metal femoral head sizes and surface replacements have been associated with edge loading, with a number of patients reporting adverse reactions to metal debris (ARMD)[4]:

- Extensive necrosis around the implant
- Pseudotumours (mass or cysts)
- Immunological responses

Ceramic bearings have also been associated with edge loading and on occasion catastrophic failure[5]. The consequences of CoCr and ceramic wear particles at the cellular level have not been fully investigated. In particular, how these wear particles interact with the cellular membrane and subsequently induce cellular responses has not been studied.

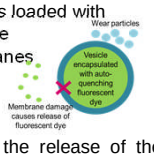
The aim of this study was to determine if CoCr and ceramic wear particles were able to damage the cell membrane with toxic consequences or whether they passed through the membrane and exert toxic effects intracellularly

Materials & Methods

Generation of wear particles: Clinically relevant CoCr wear particles were generated using a pin-on-plate tribometer. Due to the low wear of ceramic bearings, commercial alumina nanoparticles (PlasmaChem) were purchased. The wear particles were collected and imaged using FEGSEM.

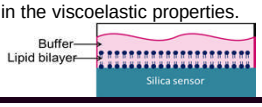
Cell viability: The effect of CoCr wear particles on the viability of L929 fibroblast cells was measured over 2 days using the MTT assay. Cells were cultured in the presence and absence of fetal bovine serum (FBS) in the presence of the particles.

Vesicle leakage assays: Vesicles loaded with an autoquenching fluorescent were formed from phospholipid membranes and exposed to 10% (v/v) FBS, followed by incremental concentrations of particles.



Vesicle leakage would result in the release of the dye, reducing its effective concentration, which increases its fluorescence, as measured over time using a fluorometer.

Quartz crystal microbalance with dissipation (QCM-D): Particle binding to a model membrane was assessed using QCM-D. A quartz crystal oscillates at its resonance frequency and changes in frequency and dissipation were measured in real-time. Binding of the particles to the solid supported bilayer induces a frequency decrease. The dissipation measures changes in the viscoelastic properties.



Results

Wear Particle Characterisation

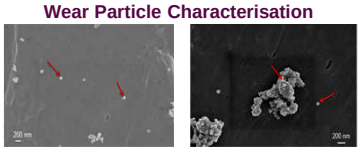


Figure 1: FEGSEM images of ceramic nanoparticles (A) and cobalt chromium wear particles (B)

The mode size of the ceramic particles was 40 nm. Cobalt chromium particles were 40-49 nm, with a round morphology. Both these size ranges fall in the size range reported in the literature[6].

Vesicle Leakage Assays

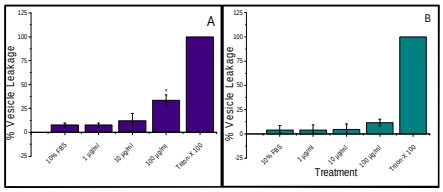


Figure 2: The effect of wear particles on vesicle leakage in the presence of serum (FBS) (A) Ceramic nanosized particles; (B) CoCr wear particles. Results expressed as a percentage of the control, detergent Triton-X 100. Vesicle leakage was measured in the presence of 10% FBS and subsequent increasing concentrations of particles. The mean (+/- 95% CL) and statistically significant results * ($p<0.05$) are shown.

The presence of FBS induced minor vesicle leakage for CoCr wear particles. Only the highest concentration (100 µg.ml^{-1}) of ceramic particles was statistically significant in comparison to the vesicles in the presence of serum only.

The effect of serum and particles on cell viability

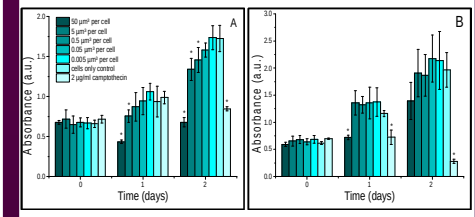


Figure 3: The effect of CoCr wear particles on L929 cell viability in the absence of 10% (v/v) FBS: L929 fibroblast cells were cultured with CoCr particles in the presence of FBS (A) and in the absence of serum (B). The mean (+/- 95% CL) and statistically significant results * ($p<0.05$) are shown.

After 2 days, the reduction in cell viability in the presence of serum and CoCr particles was greater than cells cultured with CoCr particles in the absence of serum.

Results

Wear Particle Interactions with Model Membrane

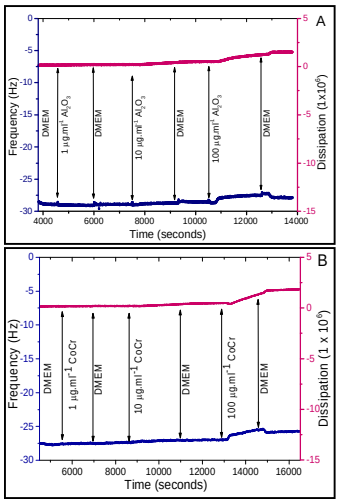


Figure 4: Assessment of particle binding using quartz crystal microbalance with dissipation: (A) Ceramic; (B) CoCr; Solid-supported bilayer lipid membranes were formed onto an oscillating quartz crystal. Incremental concentrations of particles were added followed by DMEM washes, as indicated. Changes in the frequency (blue lines) and dissipation (red lines) was measured.

No change in frequency was detected, but an increase in dissipation was measured at higher particle concentrations of 100 µg.ml^{-1}.

Effect of Serum on Particle Binding to Model Membrane

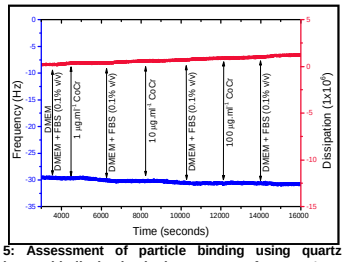


Figure 5: Assessment of particle binding using quartz crystal microbalance with dissipation in the presence of serum: An example of CoCr wear particles in the presence of serum is shown. Solid-supported bilayer lipid membrane was formed onto an oscillating quartz crystal. Upon the addition of DMEM plus 0.1% (v/v) of serum, incremental concentrations of particles were added. Changes in the frequency (blue lines) and dissipation (red lines) was measured.

No major change in frequency and dissipation were detected. Previous results have demonstrated a transient binding effect of particles in the presence of serum, however these were not repeatable as the serum itself interacted with the tethered bilayer, masking the effects.

Conclusions

- Clinically relevant CoCr and ceramic wear particles have been isolated and characterised in terms of size, morphology and biological responses.
- CoCr particles in the presence of FBS significantly adversely effected cell viability in comparison to cells cultured in the absence of serum after 48 hours. This suggests that the protein corona plays an important role in wear particle toxicity.
- CoCr particles did not induce extensive vesicle damage. Significant vesicle leakage was measured only for the highest concentration of ceramic particles, thus suggesting the loss of membrane integrity is not the main mode of particle toxicity.
- Unexpected results for the QCM-D analysis were observed: No overall particle binding was observed with only a minor change in the dissipation. Surface Plasmon resonance analysis (data not shown) confirmed that the particles do not bind to the lipid membrane. In the presence of serum, no membrane binding was detected. Therefore the changes in cell viability are unlikely to be caused by membrane toxicity. Future work will look at the incorporation of proteins into the membrane to investigate protein: particle interactions.

References [1] NJR. National Joint Registry for England and Wales: 10th Annual Report 2013. [2] Dowson. D. *et al*. 2004. J Arthroplast 19:118-123. [3] Nevelos JE, *et al*. 2001. J Mater Sci-Mater Med 12:141-144. [4] Gill HS. *et al.* 2012. Trends Mol Med 18:145-155. [5] Amanatullah. *et al*. 2011. J Arthroplast 26:72-77. [6] Brown *et al.* 2006. Proc Inst Mech Eng Part H – J Eng Med 220:355-369.

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