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A Comprehensive Assessment of Biological Responses to Silicon Nitride Nanoparticles and Cobalt Chromium Wear Debris from Total Hip Replacements

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Introduction: Silicon nitride (SiN) is an advanced ceramic that has shown potential as an orthopaedic biomaterial in its bulk form and as a coating material on cobalt-chromium (CoCr) substrates. Its high mechanical strength, low friction characteristics, low wear rates and particle dissolution characteristics are suited for next generation longer lasting total hip replacements. Moreover, SiN has been shown to have osseointegration properties. However, there is limited evidence to support its biocompatibility as an implant material. The aim of this study was to investigate cytotoxicity, inflammatory cytokine release and genotoxicity (DNA damage) of peripheral blood mononuclear cells (PBMNCs) isolated from healthy human volunteers to SiN nanoparticles and CoCr wear debris.

Materials and Methods: SiN nanopowder (<50nm, Sigma UK) and CoCr wear particles (nanoscale, generated in a multidirectional pin-on-plate reciprocator) were heat-treated for 4 h at 180°C and dispersed by sonication for 10 min prior to their use in cell culture experiments. Whole peripheral blood was collected from healthy donors (ethics approval BIOSCI 10-108, University of Leeds). The PBMNCs were isolated using Lymphoprep® as a density gradient medium and incubated for 24 h in 5% (v/v) CO_2 at 37°C to allow attachment of mononuclear phagocytes. SiN and CoCr particles were then added to the phagocytes at a volume concentration of 50 μm^3 particles per cell and cultured for 24 h in RPMI 1640 culture medium in 5% (v/v) CO_2 at 37℃. Cells alone were used as a negative control and lipopolysaccharide (LPS; 100 ng/ml) was used as a positive control. Cell viability was measured after 24 h by ATPLite assay (PerkinElmer) and tumour necrosis factor alpha (TNF-α) release was measured by sandwich ELISA (Diaclone). Results from cell viability assays and TNF-α response were expressed as mean ±95% confidence limits and the data was analysed using one-way analysis of variance (ANOVA) and Tukey-Kramer post-hoc analysis. DNA damage in the cells was measured by using alkaline comet assay (Tevigen). Hydrogen peroxide (100μm) was used as a positive control and cells alone as a negative control.

Results and Discussion: At a high volume concentration of particles (50μm^3/cell), SiN did not affect the viability of PBMNCs, while CoCr significantly reduced the viability over a 24 hour period [Figure 1A]. Additionally, CoCr particles caused significantly elevated levels of pro-inflammatory cytokine TNF-α, whereas no inflammation was associated with SiN particles [Figure 1B]. The Comet assay detected no DNA damage in cells cultured with SiN particles, whereas CoCr wear debris caused noticeable damage to the DNA [Figure 2].

Conclusion: This study has demonstrated the in-vitro biocompatibility of SiN nanoparticles with primary human monocytic cells. Therefore, SiN is a promising orthopaedic bearing material not only due to its suitable mechanical and tribological properties, but also due to its biocompatibility.

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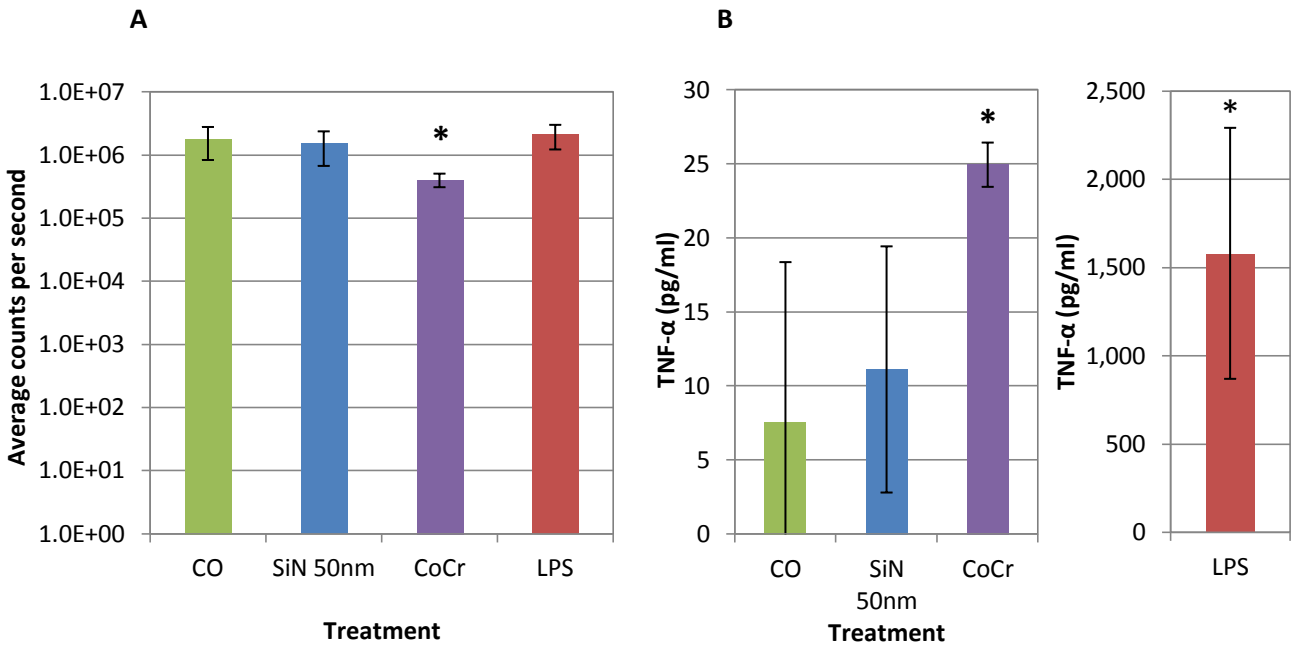

Figure 1. A) Viability of peripheral blood mononuclear cells (PBMNCs) cultured with silicon nitride (SiN) 50nm model particles and cobalt chromium (CoCr) wear debris at 50 μm^3 particles per cell. B) TNF-α release in PBMNCs cultured with SiN 50nm model particles and CoCr wear debris at 50 μm^3 particles per cell. CO: Cells only control, LPS: Lipopolysaccharide positive control. *Significant difference from the cell only control (ANOVA and Tukey-Kramer post hoc test, $p<0.05$).

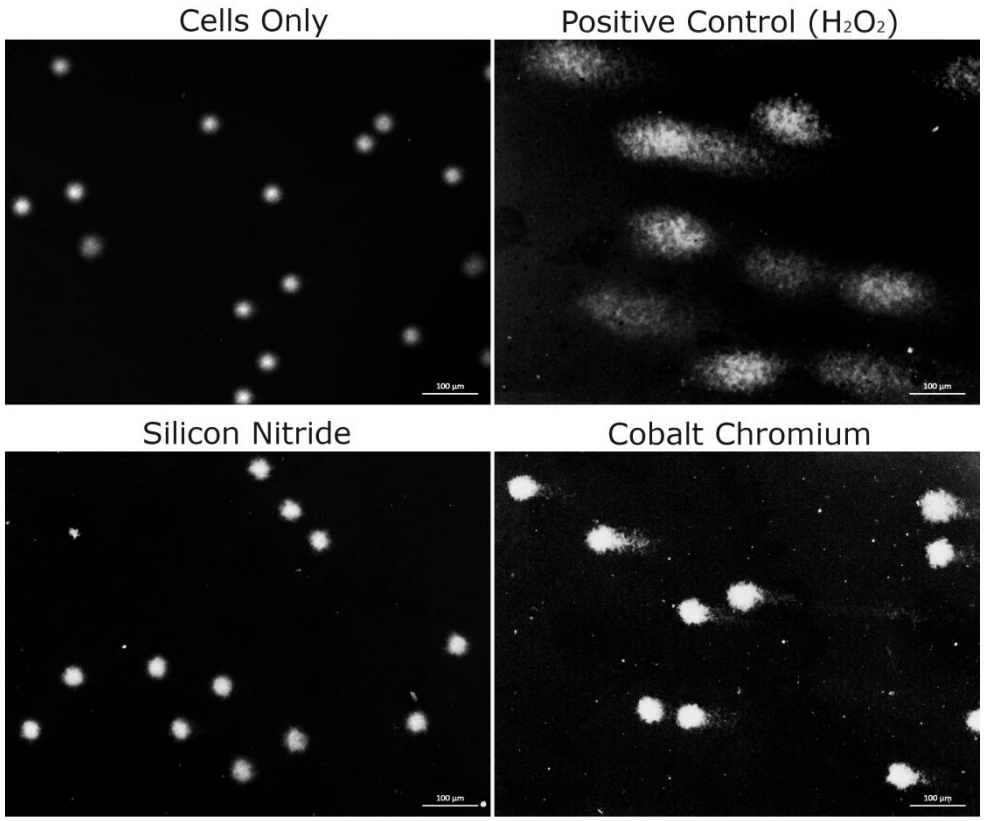

Figure 2. Single-cell gel electrophoresis images of PBMNCs. A) No noticeable DNA damage seen in cells only control. B) Extensive DNA damage seen in positive control (100μM H_2O_2). C) No noticeable DNA damage seen in cells cultured with silicon nitride 50nm model particles. D) Visible DNA damage seen in cells cultured with cobalt chromium particles.