

## Systemic Toxicity Concerns of Metal Products from Total Hip Replacements: A Perspective from A549 Lung Cells

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**INTRODUCTION:** Total Hip Replacements (THRs) represent a primary therapeutic treatment for osteoarthritis patients. Nevertheless, the advent of THRs brings forth the potential for implant-associated toxicity, triggered by the release of wear particles. These particles, in turn, can provoke adverse reactions within local tissues, orchestrated by monocytes, macrophages, and lymphocytes situated in the proximity of the implant. Such reactions can culminate in diverse complications including metallosis, implant loosening, and osteolysis, as indicated by prior research<sup>1</sup>. With the passage of time, the aforementioned wear particles may infiltrate the systemic circulation, reaching distant organs, including the lungs. Given that alveoli facilitate gaseous exchange and subsequent blood purification, comprehending the mechanism through which these wear particles infiltrate the lungs and elicit toxicity is of paramount importance. Recent investigations have demonstrated detrimental alterations within lung tissue, subsequently impacting patients' overall quality of life post-THR surgery<sup>2,3</sup>. We hypothesize lung toxicity stemming from metal debris discharged by THRs constitutes a substantial apprehension, frequently associated with the phenomenon of metallosis. In this context, the residual metal fragments engender chronic inflammation within tissues. To that end, our primary objective is to evaluate the impact of these wear particles on human adenocarcinoma alveolar basal epithelial cells (A549) through *in vitro* experimentation. By doing so, we seek to unravel the underlying mechanism driving cytotoxicity, thereby substantiating our hypothesis that wear products originating from implants hold the potential to inflict damage on lung tissue.

**METHODS: (i) Cytotoxicity assays:** A549 cells were purchased from ATCC, United States, and cultured in F-12 K media supplemented with 10 % FBS and 1% antibiotic, and the cells were maintained at 37°C in 5% CO<sub>2</sub> incubator. A549 cells were then seeded at a density of 30,000 cells/well in 48-well plates. After reaching 80-100% confluency, the cells were treated with varying concentrations (5, 20 and 50 ppm) of CoCr-P, Co I, and Cr I and incubated for 3, 7 and 14 days at 37°C. The viability of cells was measured by alamarBlue assay, the ratio of live to dead cells was measured using live-dead stain, and nuclear integrity was analyzed using DAPI staining. **(ii) Determining Cellular and DNA damage:** To understand the mechanism of action of particles and ions, A549 cells were first seeded in 24-well plates at a density of 50,000 cells/well and treated with varying concentrations of particles and ions and incubated for 3, 7 and 14 days at 37°C to study the production of Reactive Oxygen Species (ROS). The cells were then treated with 20 μM DCFDA and imaged after 45 minutes using a fluorescence microscope. Consequently, to analyze the gene expression through RT-PCR, A549 cells were seeded in 12-well plates at a density of 1\*10<sup>5</sup> cells/well and treated with varying concentrations of particles and ions and incubated for 3, 7, and 14 days at 37 °C. The cells were then incubated with 250 μl Trizol reagent and RNA isolation was performed, followed by cDNA synthesis and qPCR analysis for three DNA damage specific genes: GADD45A, XRCC1, PCNA, with GAPDH as a housekeeping gene. Finally, A549 cells were seeded in 6-well plates at a density of 1\*10<sup>6</sup> cells/well and treated with varying concentrations of particles and ions, and incubated for 3, 7, and 14 days at 37 °C to analyze Apoptosis/Necrosis in the cells using flow cytometry. The cells were then collected and washed with ice-cold PBS and stained with Annexin-V conjugated with FITC and PI and analyzed through a flow cytometer. **(iii) In vivo animal studies - Histopathology of the lung tissues:** C3H/HeJ Mice were treated with 50 ppm CoCr-P through intraarticular injection. After 45 days, the mice were euthanized, and H&E staining of mouse lung tissue was performed.

**RESULTS:** The cell viability was successfully assessed using the alamarBlue assay. A549 cells exhibited the lowest fold cell viability for CoCr-P, with a significant decrease in viability observed from 30 ppm treatments on day 3 (Fig A-i). On days 7 and 14, A549 cells demonstrated significant results from 5 ppm across all treatments, with Cr I exhibiting the lowest fold cell viability (Fig A-ii&iii). These findings were supported by DAPI staining (Fig B - (i-iv)) and live-dead (Fig C - (i-iv)), showing a significant decrease in the number of live cells and a reduction in fluorescent intensity, respectively. In the ROS assay (Fig D - (i-iv)), Cr I ions exhibited significantly higher fluorescence with an increase in concentration and time-points, compared to CoCr-P and Co-I. Initially, CoCr-P demonstrated higher ROS production than the ions. A549 cells also displayed an upregulation of GADD45A, XRCC1, and PCNA, indicating DNA damage upon exposure to particles and ions. Upon visualization of H&E staining of mouse lung tissue, it was observed that mice treated with CoCr-P displayed thickened alveolar walls and the presence of macrophages and neutrophils in the alveolar space, indicative of inflammation resulting from particle exposure. Statistical analyses were conducted using GraphPad Prism Software, employing ordinary two-way ANOVA followed by a Tukey post hoc test. A significance level of  $p < 0.05$  was adopted for the interpretation of results.

**DISCUSSION:** This study confirms that CoCr-P, Co I, and Cr I have dose- and time-dependent cytotoxic and genotoxic effects. CoCr-P caused immediate cellular and DNA damage, whereas Co I and Cr I had gradually more significant impacts on cells. The ROS test revealed elevated ROS generation in response to ions and particles, which raises the possibility of ROS-induced cell death. These findings were further corroborated by the upregulation of genes related to DNA damage. Histopathological analysis of mouse lung tissue revealed that the migration of these particles and ions to the lungs caused inflammation and cell death. These results underline how important it is to understand the systemic toxicity that might result from metal fragments in total hip replacement (THR), particularly regarding lung cells. This research will be extended to study the underlying processes and create efficient mitigation techniques to increase metal implants' safety in THRs. In the end, this research offers insightful information for minimizing possible risks associated with metal debris, directing the development of THR technologies, and enhancing patient well-being.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Healthcare providers may use our data to help them make more informed choices about THR patients and the possible risk of toxicity from implant-generated wear particles on lung tissue.

**REFERENCES:** [1] Kolat et al., 2015, [2] Zhan et al., 2019, [3] T Balbouzis et al., 2016.

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**Figure 1:** The major findings of the study:

