Effects of Implant Wear Products on Myoblasts and Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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INTRODUCTION: In the United States, an estimated 450,000 total hip replacement (THR) surgeries are carried out each year, and is anticipated to rise by 167% by 2040[1]. THR surgery is an available therapy method for patients suffering from osteoarthritis (OA), although the long-term efficacy of this intervention remains uncertain. THR implants provide a solution for individuals experiencing joint-related issues; because they are composed of diverse metal compositions (cobalt, chromium, titanium, and molybdenum), these implants are susceptible to tribocorrosion. During this process, implants endure mechanical and chemical deterioration, resulting in the release of degradation products (DPs) such as metal ions, metal particles, metal-protein complexes, and inorganic metal oxides[3,4]. Localized effects of DPs have been extensively studied, but little is known about how these DPs affect systemic organs, particularly the cardiac tissue. In 2013, a case study reported a female patient, aged 58, who had previously undergone metal-on-metal (MoM) resurfacing THR surgery a decade ago presented symptoms of heart failure[5]. Further examinations of blood serum indicated high levels of Co ions 169 ppb and Cr ions 31 ppb, past the acceptable range of 7 ppb. Therefore, the objective of the current study is to investigate the potential toxicity of cobalt-chromium-molybdenum particles (CoCrMo particles), cobalt ions (Co ions), and chromium ions (Cr ions) on cardiac cells and mice model. We hypothesize that the aforementioned degradation intermediates may be detrimental to cardiac functions. The following are among the aims of the study: (i) To examine possible morphological changes and toxic consequences by exposing myoblasts cells (H9C2) to DPs under both static and dynamic environments (ii) To investigate the side effects of DPs to induced pluripotent stem cells (iPSCs) derived cardiomyocytes. (iii) To investigate the histopathological impact on cardiac tissue in an animal model following an injection of DPs, where tissue samples were collected after 45 days.

METHODS: (i) Degradation products (DPs): CoCrMo particles are sourced from Bioengineering Solutions, Chicago. Co ions are obtained from ACROS Organics, Belgium. Cr ions are procured from Alfa Aesar (Thermo Fisher). (ii) Cell lines: H9C2 were purchased from the American Type Culture Collection (ATCC). Feeder-dependent normal WT1-iPSCs were established previously using an episomal method. (iii) Static cell viability assays: The following cell viability assays were performed on the H9C2 control cell line for 1, 3, 5, and 7 days: Alamar blue, Live/Dead staining, DAPI, and ROS. For Alamar Blue, DAPI, and Live/Dead assays, cells were seeded on 48-well plates. For ROS assay, cells were seeded on 24-well plates. Treatment was commenced upon cell adherence and confluency per well. The treatments were Co ions, Cr ions, and CoCrMo particles, with concentrations of 0.25 ppm, 0.5 ppm, 1 ppm, 5 ppm, 10 ppm, 20 ppm, and 50 ppm. Each experiment was performed in triplicates as per respective protocols. (iv) Dynamic cell viability assays: H9C2 cells were seeded onto a glass slide and subsequently fixed onto silicone channels, which formed a microfluidic chamber. The connection to a peristaltic pump allows for dynamic culturing conditions, enabling the constant media flow to the H9C2 cells. DAPI was conducted according to the protocol in duplicates after one day, with a concentration of 20 ppm being selected. (v) Gene expression analysis: H9C2 control cells were seeded on 12-well plates for 1 and 3 days in triplicates per concentration of treatment. Cell treatment began upon full confluency of cells. RNA was isolated, then chosen genes were amplified via RT-PCR, and their expression was measured via qPCR. The amplified and quantified genes were GAPDH (housekeeping gene), Cox62a (metabolism gene), GATA4, and PDE4A. (vi) WT1-iPSCs differentiation to cardiomyocytes: Cells were differentiated per protocol[6]. (vii) Histopathological study: Control and treatment groups of six mice [C3HeJ/000659, IACUC ID: 1684850-3] were subjected to 10 µl of 50ppm CoCrMo particle injection. After forty-five days, groups were euthanized, and heart organ biopsies were placed in formalin. The cardiac tissue specimens underwent cross-sectional slicing and were placed in an Optimal Cutting Temperature solution. Frozen biopsies were sectioned via cryostat following protocol and imaged via standard bright field techniques.

RESULTS: From the results, CoCrMo particles are more toxic than Co and Cr ion treatments and control, even at lower concentrations and shorter exposure times. Using **Figure A**, on the first day of exposure, H9C2s subjected to CoCrMo particles at a concentration of 0.25 ppm exhibited a cell viability of 93.18%. At the higher concentration of 50 ppm, cell viability experienced a significant decrease, resulting in a 64.12% cell viability. Notably, Cr ions exceeded Co ions in terms of cytotoxicity. After seven days, lower concentrations of 0.25 ppm Cr ions (86.02% cell viability) and Co ions (84.90% cell viability) showed significant toxicity. The degree of toxicity increased over time. Results from Live/Dead image staining, consistent with the cell viability data, support this discovery. Additionally, **Figures B and C** show gradual declines in cell viability as CoCr P concentration increased. **Figure D** shows increased ROS per increase in CoCr P. The altered expression of Cox6a2 shown by the RT-PCR gene expression analysis suggested possible abnormalities in cell cycle, contractility, and metabolism (**Fig E**). Additionally, we observed harmful effects and nuclear integrity impairment linked to CoCrMo particles using a sophisticated microfluidics simulator. Created feeder-independent WT1-iPSCs successfully differentiated into functional cardiomyocytes by virtue of their contractility (**Fig F**). Preliminary H9C2 data indicate possible changes in viability, gene expression, and morphology despite the lack of study on these cells. Finally, a mouse model exhibited little to no morphological alterations in sectioned cardiac tissue, signifying that CoCrMo particles did not reach cardiac tissue (**Fig G**).

DISCUSSION: Based on our initial findings, it appears DPs may have an adverse effect on cardiac cells. However, further studies are required to understand the underlying mechanism. We speculate, using **Figure H** as a diagram, that when DPs react with mitochondria, they generate reactive oxygen species (ROS), inhibiting cellular respiration[7]. ROS can potentially infiltrate the nucleus and cause DNA damage[7]. This study has several limitations, as it is *in-vitro* high dosimetry, short exposure simulation, and far away from *in-vivo* conditions. In the future, we plan to develop the H9C2 into a more cardiac-like phenotype. This will help better mimic the toxic effects of implant-derived products on cardiomyocytes. One of the notable accomplishments of our group is the effective differentiation of WT1-iPSCs into cardiomyocytes. We believe that with protocol improvements, cardiomyocyte differentiation will be more productive.

SIGNIFICANCE: This work provides insights into the impact of DPs-induced cardiomyocyte damage. Limited research on this subject makes the findings more clinically significant and underlines the importance of stem cell-based research in understanding the adverse effect of DPs on vital organs.

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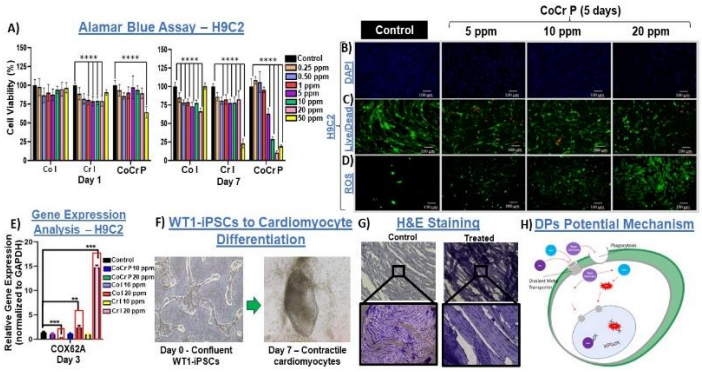

Figure: Cardiac cell assay results:
(A) Alamar blue demonstrated CoCrMo particles had the most toxicity, Cr ions second-most, and Co ions least.
(B) DAPI assay supported the results of the Alamar blue assay.
(C) Live/Dead assay supported the results of the Alamar blue assay.
(D) ROS assay supported the results of the Alamar blue assay.
(E) RT-PCR analyses demonstrated a lower expression of Cox62a for CoCr particles, which is associated with mitochondrial dysfunction. The higher expression of Co ions and Cr ions is associated with inflammation.
(F) Successful differentiation of WT1-iPSCs to contractile Cardiomyocytes
(G) H&E staining of control and treatment heart sections showed little to no alterations.
(H) DPs react with the mitochondria to inhibit cellular respiration and induce ROS.