Toxicity Risk Associated with Hip Implants on Obese Population: Cobalt Ions on Systemic Adipocyte Function

Katherine Elbert[1], Rukshini Venkatesan Bharathi[1], Mareeswari Paramasivan[1], Abeer Mohamed[2]; Mark Barba[3]; Mathew T. Mathew[1]
[1]*RMDR Lab, UIC College of Medicine Rockford, Rockford, IL;* [2]*Department of Medicine, UIC College of Medicine, Chicago;* [3]*Orthoillinois, Rockford, IL*
kelbe@uic.edu; mtmathew@uic.edu
Disclosures: K. Elbert (N), RV. Bharathi (N), M. Paramasivan (N), A. Mohamed (N), M. Barba (N), MT. Mathew (N)

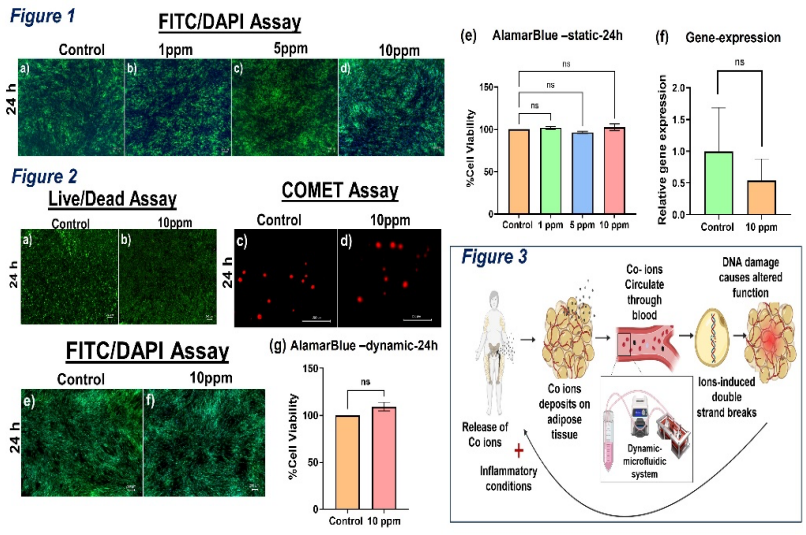
INTRODUCTION: Osteoarthritis (OA) is a leading cause of disability worldwide and affects over 528 million individuals[1], and has grown by over 110% in the past three decades[2]. Obesity (BMI >30 kg/m^2) accelerates OA progression through mechanical loading, inflammation, and metabolic dysfunction, leading to earlier joint replacement[3,4]. Total hip arthroplasty (THA) is an effective intervention for end-stage OA, and approximately 544,000 THAs are performed annually in US[5]. However, outcomes are often poorer in obese individuals due to elevated risks (4 times) of perioperative complications and implant failure[6]. A potential concern in THA is the release of metallic debris, especially cobalt ions, as these implants are made of CoCrMo alloys. It is well reported that these ions can interact with local and systemic tissues, potentially exacerbating inflammation and contributing to adverse outcomes[7]. Despite the known inflammatory role of adipose tissue in obesity and OA, little research has explored how cobalt ions affect adipose tissue function. Since adipose tissue is vascularized, it is plausible that circulating cobalt ions can systemically affect adipose tissue function, especially in the context of obesity. Most of the studies are in static conditions (ions are added directly to cells), but by using an advanced microfluidic system, dynamic (media is circulating) conditions can be achieved, which may better mimic systemic circulation and physiological interactions. Therefore, this study addresses the possible toxicity risk of cobalt ions on adipose tissue function by evaluating the inflammatory and genotoxic effects on adipocytes under static and dynamic culture conditions. We hypothesize that dynamic culturing more closely simulates in vivo physiology, and that cobalt ion exposure will induce measurable alterations in adipocyte function.

METHODS: (a) Static culturing: Human Adult Subcutaneous Preadipocytes acquired from Cell Applications, Inc. were grown in Preadipocyte Media from Cell Applications, Inc. and seeded at a density of 1x10^6, 50,000, 30,000 cells/well in 12, 24, and 48 well plates, respectively, at 37°C in a 5% CO_2 incubator. The preadipocytes were then differentiated with Adipocyte Differentiation Medium from Cell Applications, Inc. **(b) Dynamic Microfluidic System:** Human Adult Subcutaneous Preadipocytes were seeded at a density of 1x10^5 cells/well and grown on glass slides with silicone chambers. Once the adipocytes were fully differentiated, the glass slides were transferred to the dynamic microfluidic test system in an incubator and exposed to 10 ppm of cobalt ions (cobalt II chloride purchased from Acros Organics) at a previously validated flow rate of 0.2 ml/min[8] to simulate metal particle release from orthopedic implants **(Fig. 3).** Cells were imaged every 30 minutes during the 24-hour study to observe changes in cell morphology and lipid accumulation under treated conditions. **(c) Cytotoxicity assays:** Cellular integrity imaging was carried out using FITC-DAPI staining. Viability was quantified using an Alamar Blue assay. **(d) Genotoxicity assays:** The control and treated cells were reverse transcribed using RT-PCR and quantified for Caspase-9 gene using qPCR with GAPDH as a housekeeping gene. The comet assay was used to visualize and quantify DNA strand breaks in the adipocyte nuclei to evaluate potential DNA damage.

RESULTS: Cytotoxicity assays confirmed that cobalt ion exposure up to 10 ppm did not significantly impair adipocyte viability at 24 hours under static conditions in FITC/DAPI **(Fig. 1a- d),** Alamar Blue **(Fig. 1e),** and Live Dead assays. Because there was more cell viability observed in the treated condition, the apoptosis gene Caspase-9 was analyzed to determine if apoptosis was upregulated or downregulated when treated with metal ions. The qPCR results correlate with this finding of decreased apoptosis, showing a decrease in Caspase-9 gene expression (**Fig. 1f**). Based on these findings, 10 ppm was selected as the maximum exposure concentration for dynamic culture experiments. Adipocytes exposed to 10 ppm cobalt ions under dynamic conditions for 24 hours maintained high cell viability, with no significant cytotoxicity observed on Live Dead **(Fig. 2a- b),** FITC/DAPI **(Fig. 2e- f),** and Alamar Blue assays **(Fig. 2g)**. This suggests that cobalt ion exposure, at least in the short term, does not impair metabolic activity or overall cell survival. However, comet assay analysis revealed increased tail-like projections, suggesting genotoxicity with increasing DNA strand breaks **(Fig. 2c- d)**. Also, lipid accumulation was observed throughout the 24-hour study in the treated cells as seen on continuous cell imaging. The adipocytes may be protected from cobalt ions by lipid production, limiting the cytotoxic effects of cobalt.

DISCUSSION: The study shows the hypothesis was validated with dynamic culturing, more accurately simulating systemic circulation. Cobalt ion exposure did not produce overt cytotoxicity in adipocytes under either static or dynamic conditions, but it did induce genotoxicity. The absence of cytotoxicity demonstrates that cobalt ions are not acutely lethal to adipocytes, yet the observed genotoxicity points toward subtler long-term consequences. The accumulation of lipids throughout dynamic culturing needs further exploration, as this observed finding could be a cell-mediated protection mechanism from the stress of cobalt ions. The DNA strand breaks in adipocytes may contribute to dysregulated adipokine secretion, premature senescence, or pro-inflammatory signaling, all of which are central to the metabolic dysfunction seen in obesity. Importantly, the finding that dynamic conditions amplified genotoxicity shows the physiological relevance of flow-based models. Static culture provides a useful baseline, but may underestimate systemic exposure, as diffusion alone does not capture the vascularized, circulating environment of adipose tissue. Dynamic models replicate this circulation, showing that cobalt ions, once mobilized into the bloodstream from implant wear or corrosion, may exert systemic effects on adipose tissue. This is especially significant in obesity, where expanded, inflamed adipose tissue already contributes to a pro-inflammatory state. Our results suggest that adipose tissue may represent an additional systemic target for implant-derived metal ions. In obese patients, the combination of expanded adipose volume, chronic low-grade inflammation, and heightened susceptibility to genomic injury could exacerbate implant-related complications, including impaired healing, systemic inflammation, or even accelerated implant loosening. Limitations include the simplified in vitro setup and absence of immune cell culture, which may influence inflammatory signaling. Future research is required to explore genetic and protein variations in lipid metabolism.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings offer a new perspective on implant-induced adipose tissue dysfunction in THA patients with obesity who may already have heightened inflammatory and metabolic vulnerability. Also, the demonstration that dynamic culture conditions more accurately reflect systemic circulation and tissue-level responses emphasizes the necessity of such models in implant toxicity testing, highlighting the need for personalized orthopedic approaches to improve outcomes for obese patient populations.



REFERENCES: [1] Osteoarthritis, WHO, 2019. [2] Osteoarthritis Action Alliance (2025) [3] Coggon et al. 2001 [4] Guenther et al. 2015 [5] Joint Replacement Surgery. [6] Katakam et al. 2021 [7] Bonnani et al. 2023 [8] Badhe et al. 2021.

ACKNOWLEDGEMENTS: Blazer Foundation, Rockford; Office of Research, UICOMR

Fig. 1: a-d) Static Culturing for 24 h FITC/DAPI Assay at 1, 5, and 10 ppm. **e)** Static Culturing for 24h Alamar Blue Assay. **f)** qPCR for apoptosis gene. **Fig 2: a-b)** Dynamic Microfluidic System for 24h Live/Dead Assay. **(c-d)** Dynamic Microfluidic System for 24h COMET Assay.**e-f)** Dynamic Culturing for 24 h FITC/DAPI assay at 10 ppm. **g)** Dynamic system for 24h Alamar Blue Assay **Fig 3:** Schematic diagram showing mechanism of cellular damage