

# An *in-vitro* study on the Toxicological Effect of Metal Products on Adipose tissue: Obese Population with Hip Implant

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**Introduction:** Nearly 70 million people were affected by arthritis in the US by 2024, the leading causes being age, **obesity** (BMI over 30Kg/m<sup>2</sup>), lifestyle, and genetic factors. <sup>1</sup> According to the CDC data, almost 31% adults with obesity are diagnosed with arthritis. <sup>2</sup> This directly increases the need for Total Hip Replacement (THR) surgeries in obese people. <sup>3</sup> THR components (femoral stem/head) are made of metal alloys (CoCrMo, T6Al4V). Further, the overweight may lead to complications after a total hip arthroplasty and have a negative impact on prosthesis longevity. <sup>4</sup> The overall revision rate is 7.99% higher for obese patients when compared to the normal population, which means that due to the increase in load and other functional activities, there is an increase in friction and wear (tribocorrosion) at implant interfaces, causing the release of metal products to the joint environment. <sup>3</sup> Literature extensively reported the potential risk of an adverse tissue reaction, local toxicity, and systemic toxicity due to the metal particles/ions. <sup>6,7</sup> Mostly, the hip implants are surrounded by subcutaneous adipose tissue that is neutral and protective, which is primarily exposed to the degraded particles from the implants. <sup>5</sup> These tissues are mainly responsible for metabolic function, hormonal and lipid homeostasis, and energy storage. However, there are only limited studies on the toxicity of metal products on adipose tissue. We hypothesize that in obese patients, the metal products will react with the adipocytes, may interrupt the function, and cause other side effects. Hence, the objective is to study the cytotoxicity and genotoxicity of metal ions (Co, Cr, Ti) with different concentrations and time periods on the adipocytes.

**Materials and methodology:** Human preadipocytes (HPad) were obtained from Cell Application, Inc. and were differentiated into adipocytes. Cultured the pre-adipocytes in a T75 flask using Preadipocyte media from the Cell Application, Inc. at 37°C in a CO<sub>2</sub> incubator. Standard solution with metal ions (Co, Cr, Ti) purchased from Acros Organics, Thermo Fisher Scientific, Sigmaaldrich as Cobalt (II) Chloride, Chromium (II) Chloride, Titanium standard solution, respectively. **Cytotoxicity:** Seeded 30,000 cells/well in a 48-well plate using preadipocyte media and differentiated using adipocyte differentiation media (Cell Application, Inc.) to study the quantitative analysis of cell viability assay (Alamar blue). To qualitatively analyze the cell viability and nuclear integrity using live/dead and FITC/DAPI, 50,000 cells/ well were seeded in a 24-well plate. A 6-well plate was seeded with 300,000 cells/well to study the cell death mechanism using the Apoptosis/Necrosis assay using BD FACSymphony™ flow cytometry. After the seeded cells reached 80% confluency, the preadipocyte media were changed to differentiation media until all the cells were completely differentiated. The differentiated cells were treated with 1ppm, 5ppm, and 10ppm of Co, Cr, and Ti for 24 hours, 3days, and 5 days. The lab protocol was followed, and the analysis was performed. **Genotoxicity:** To check whether the metal ions cause genotoxicity to the adipocytes, 100,000 cells/well were seeded in a 12-well plate. After differentiation, the cells were treated with 10ppm metal ions (Co, Cr, Ti) for 5 days. The DNA damage was analyzed using the COMET assay. Further, to explore the growth of the adipocytes after treatment with 10ppm of (Co, Cr, Ti) for 5 days, 300,000 cells/well were seeded and differentiated, qPCR was performed by reverse transcribing the adipogenesis (BMP2) and ROS (BIP) genes, using RT-PCR and quantifying the adipogenesis gene with respect to the GAPDH gene using qPCR.

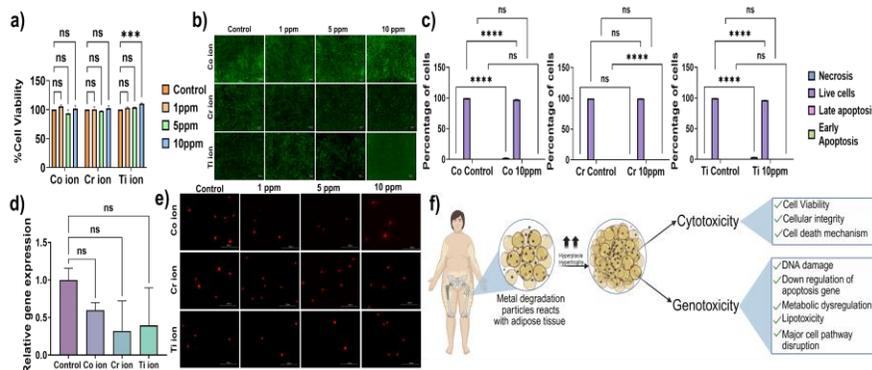
**Results: Cytotoxicity:** The Alamar blue analysis showed 99% viability of mature adipocyte cells when treated with ions (Co, Cr, Ti) with different concentrations of 1ppm, 5ppm, 10ppm for 24H, 3 days, 5 days, but in 5ppm we were able to find a decrease in cell viability in all the ion treatments. The same trend was followed in all the ions in different time periods (**Fig. a**). The qualitative analysis from the live-dead assay showed the maximum cells alive when treated with ions (Co, Cr, Ti) with the same concentration and time period. The cell viability decreased when the concentration and time increased (**Fig. b**). To check whether the ion treatment is affecting the cytoskeleton and nucleus integrity, FITC/DAPI was analyzed, and we observed no significant damage to both the cytoskeleton and nucleus of mature adipocytes. Since some dead cells were found in the primary cytotoxicity assays, an Apoptosis/ Necrosis assay was performed to check which pathway the cells undergo when treated with 10ppm ion (Co, Cr, Ti). We found that the cells live after treatment, few were observed in the necrosis pathway, and only 0.5% of cells died through the apoptosis pathway (**Fig. c**). **Genotoxicity:** Since more live mature adipocytes were observed, we wanted to check the gene expression of the ROS gene and observed a significant decrease in the BIP gene (**Fig. d**) when the cells were treated with metal ions of higher concentration 10ppm for 5days. The COMET assay was performed to check if any DNA breaks occur when the cells are exposed to metal ions. A tail-like projection caused by the damaged DNA, indicating that DNA damage was happening when the cells were treated with metal ion concentrations of 5ppm, 10ppm for 3 days and 5 days of exposure time (**Fig. e**).

**Discussion:** Our results showed that the metal products from the implants are causing no significant cytotoxicity; however, some early indication of genotoxicity to the adipocytes. So the hypothesis is partially validated. The qPCR analysis of ROS gene expression also supports the above statement, where the BIP gene is downregulated, so no reactive oxygen species and no stress will be caused to the endoplasmic reticulum when the cells are treated with metal ions of higher concentration. Remarkably, we also observed the DNA damage caused when the mature adipocytes are treated with high concentrations of 5ppm and 10ppm metal ions. This indicates that the DNA level disruption might cause gene mutation and disrupt normal functions in maintaining metabolic and hormonal homeostasis of adipose tissue in human beings. In obese people with implants, the generated metal products will be higher, which may increase the risk of other side effects and obese related complications. The study has many limitations (lab scale study, only limited dosimetry, and short duration) and further studies are required. In the long term research required to focus to develop the cell therapies to overcome DNA damage and promote the DNA repair mechanism, to avoid the risk of gene mutations and cancer. So, in our own future work we will consider to check if any metabolism-related gene expression occurs when the cells are treated with metal ions using the gene profiling technique, and analyze whether any proteins that are majorly involved in the cell signaling pathway are deregulated (**Fig. f**). This study might lead us to better understand how adipocytes tackle the metal ions leached from the implants.

**Significance:** This study indicates the possible risk of toxicity caused by metal ions on the adipose tissue of obese patients with hip implants. Understanding the toxicity mechanism, pathways, and genetic mutation assists us in formulating better approaches to next-generation safer implants in orthopedic health care.

**Reference:** [1] Kulkarni, 2016. [2] Team, S. Arthritis statistics, 2021. [3] Barrett, M, 2018. [4] Farmakis IK, 2019. [5] Björntorp, P, 2000. [6] Bijukumar, D. R, 2018. [7] Zywił, M. G, 2016.

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**Figure 1:** (a) Alamar blue assay to quantitatively check cell Viability (Co, Cr, Ti of 1, 5, 10ppm for 5 days) (b) qualitative analysis for cell viability (Co, Cr, Ti of 1, 5, 10ppm for 5 days), (c) flow cytometry analysis for Cell death mechanism (Co, Cr, Ti of 10ppm for 5 days), (d) BIP (ROS gene) regulation using qPCR analysis (Co, Cr, Ti of 10ppm for 5 days), (e) COMMET assay to check the DNA damage (Co, Cr, Ti of 1, 5, 10ppm for 5 days), (f) an overview of how metal ions impact the adipose tissue in obese patients with implants