Mitigating Corrosion and its Side Effects from Total Hip Replacement (THR) Using Antioxidants

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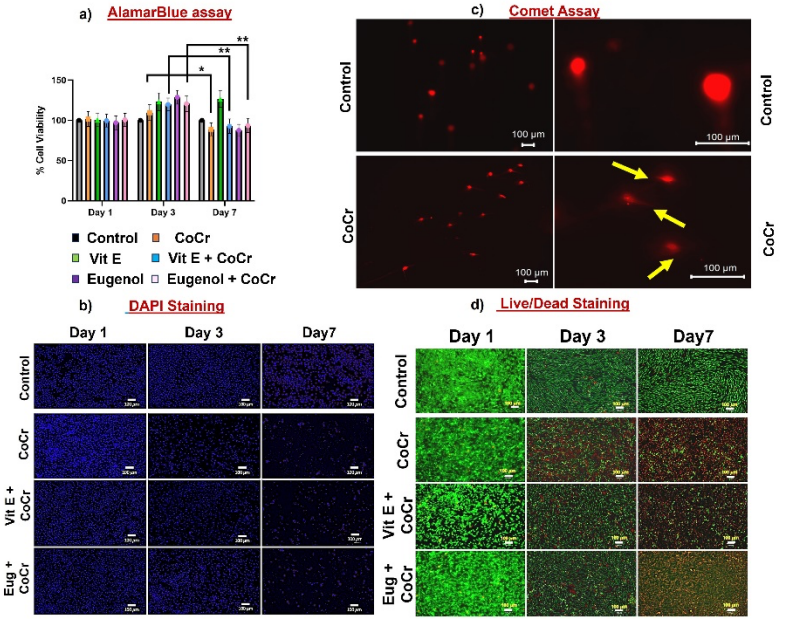
INTRODUCTION: Total Hip Replacements (THRs) have significantly contributed to the improvement of life expectancy, mobility, and independence for persons with hip problems [1]. Although total hip replacement (THR) is considered the most advantageous treatment for those with joint-related impairments, new studies have revealed that the release of particles associated with the wear and tribocorrosion of THRs might potentially cause damage to the surrounding bone and implanted materials. These particles elicit both local and systemic damage, hence requiring revision operations for patients. Considerable scientific research has been conducted to understand the effects of organic and synthetic corrosion inhibitors on the long-term durability of THRs. The inherent ability of naturally existing antioxidants to alleviate the impact of wear particles is attributed to their polar interactions with nitrogen, oxygen, and/or sulfur-free radicals. Recent research in our lab indicates that antioxidants might be considered a feasible strategy to prevent corrosion in hip implants, owing to their aforementioned characteristics and easy accessibility [2,3, 4]. In order to empirically evaluate this hypothesis, we have formulated two specific aims: (i) to assess the impact of wear particle toxicity on osteoblasts in a static environment, and (ii) to evaluate the potential of Vitamin E and Eugenol in mitigating the cytotoxic effects of wear particles through *in vitro* cytocompatibility assays utilizing osteosarcoma (MG-63) cells.

METHODS: CoCrMo particles were purchased from Bioengineering Solutions in Chicago. SEM and EDAX analyses were used to characterize the morphology of the particles [2]. In order to prevent particle agglomeration, sterile DI water was used to create a stock solution of CoCrMo particles at a concentration of 1 mg/ml. The prepared stock solution was used for conducted experiments. MG-63 osteoblast-like cells were used in static cell culture to conduct *in vitro* cytocompatibility assays to determine the localized toxicity of cobalt-chromium (CoCr) particles at concentration of 20 ppm and exposure times (1-7 days). Based on the results of our prior laboratory work, the optimal antioxidant content of 2.5 ppm was established [3]. 20 ppm CoCr particles, 2.5 ppm antioxidant concentrations (made by preparing them in inadequate minimum essential medium (MEM)), and a mixture of both particles and antioxidants were all applied to MG-63 cells. AlamarBlue, Comet, and Live/Dead tests were performed during treatment intervals ranging from 1 to 7 days to determine the viability of the cells. GraphPad Prism software was used for the statistical analysis of all experiments, which were performed in triplicates. The significance level for the one-way ANOVA, posthoc Tukey's test, and Sidak test was set at $p< 0.05$.

RESULTS: From our previous lab results of the DPPH assay, % Radical Scavenging Activity (RSA) is 120-140% at 2.5 ppm concentration was observed for the antioxidants; Vitamin E and Eugenol. Results of the alamarBlue assay conducted on cells treated with 20 ppm CoCr particle concentrations, antioxidants, and mixed solutions demonstrated no significant reduction in cell viability after the Day 1 timepoint in all groups. For longer time points, there was a significant ($p<0.05$) amount of cell viability for MG-63 cells treated with 20 ppm CoCr particle-2.5 ppm of Vitamin E and Eugenol mixtures at Day 3 and Day 7 in comparison to cells treated only with 20 ppm CoCr particle (**Fig. a**). These results were corroborated with DAPI (**Fig. b**) and Live/Dead staining images (**Fig. c & d**). DAPI Images for 20 ppm CoCr particle-2.5 ppm of Vitamin E and Eugenol mixtures on Day 3 and Day 7 show increased fluorescence and maintained nuclear integrity in comparison to the images for cells treated with just 20 ppm CoCr particle. In addition, MG-63 cells treated with both CoCr particles and antioxidants maintained a greater number of live cells and a reduced number of dead cells on Days 3 and 7 in comparison to cells treated only with CoCr particles. Comet assay was then performed to visualize DNA toxicity and the images demonstrated DNA damage to cells treated for just one day with CoCr particles (**Fig. b**).

DISCUSSION: *In vitro* cytocompatibility assays characterized by static conditions, the utilization of DAPI and Live/Dead tests enabled the observation of a decrease in cellular viability when subjected to prolonged treatment periods with 20 ppm CoCr particles. The aforementioned findings is substantiated by the utilization of imaging acquired using the Neutral Comet Assay, which effectively demonstrated the presence of DNA damage inside the cellular structures. The underlying mechanism hypothesized to be responsible for this detrimental effect involves generating free radicals due to cellular exposure to particulate matter. In addition, it was shown that antioxidants increased cell viability when subjected to extended treatment durations. When comparing samples that were treated just with particles to those that included antioxidants, it was seen that the addition of antioxidants resulted in a significant improvement in cell viability (**Fig. a**). This was confirmed by using the Sidak Multiple Comparisons Test, particularly when the incubation period was extended. The group that received antioxidant treatment demonstrated increased cell viability in the DAPI staining analysis following dynamic cell culture, which supports the findings obtained from the alamarBlue experiment. The proposed method facilitates establishing a protective layer through the action of antioxidants. These antioxidants bind to and inhibit the synthesis of corrosive wear particles, therefore impeding the generation of harmful reactive oxygen species. Nevertheless, it is crucial to acknowledge the limitations of the research. Initially, the tests were conducted using a singular concentration of antioxidants and a CoCr particle solution. Furthermore, the precise etiology of cellular injury remains unclear. In order to augment the comprehensiveness of the next study, we will perform Reactive Oxygen Species (ROS) tests on the cells and thereafter employ comet assays at subsequent time intervals to confirm the DNA damage due to the particle exposure.



SIGNIFICANCE: In response to the physiological environment that metal ions and particles may be generated from hip implants leading to both local and systemic side effects. Our findings demonstrate that antioxidants (Vitamin E and Eugenol) are a possible effective way to mitigate implant corrosion, opening a possible path for reducing such risks on the orthopedic patients.

REFERENCES: [1] AJRR 2021, [2] Manjunath, V *et al* (2021), [3] Kamaraj et al ORS 2023, [4] Chraka, A *et al* (2020)

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Figure: a) AlamarBlue Assay: Cell viability in the presence of particles, antioxidants, and mixed solutions in a static environment. **b) Comet Assay** showing DNA damage to cells treated with CoCr particle solution **c) DAPI staining:** Variations of nuclear integrity corroborating with alamarBlue assay **d) Live Dead Staining:** Analyzing the amount of live and dead cells by assessing membrane integrity