Minimizing the toxic effects due to implant wear products: Evaluating the molecular mechanisms of antioxidants using an in vivo zebrafish model

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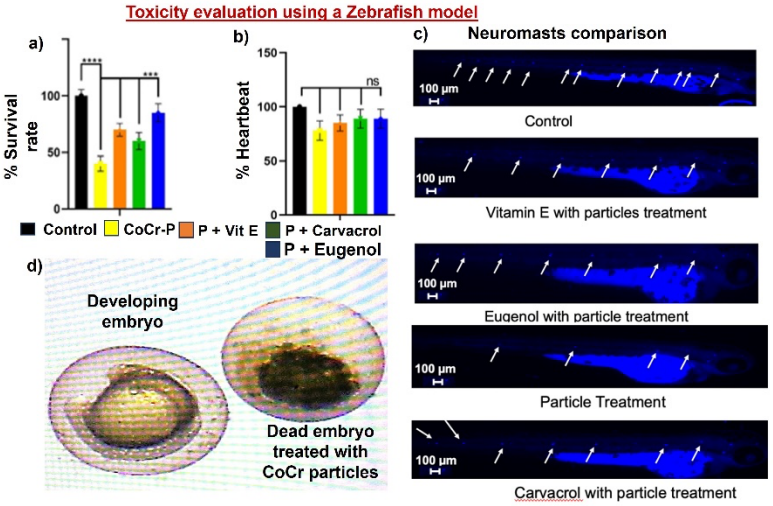
INTRODUCTION: The increasing usage of implants in orthopedics raises concerns about the toxicology aspects of implant-generated products. In toxicology research, rabbits, guinea pigs, hamsters, rats, and mice are the most popular live animal models. Nevertheless, one animal model that stands out as being particularly useful is the zebrafish. Impressively, zebrafish have around 70% of the human gene pool, and the two species share many important pathways that control the growth of these structures such as, muscle, blood, kidney and eyes. As a result, zebrafish may be used as a model to investigate illnesses that affect various systems of the human body. Zebrafish have been tremendously helpful in the study of development, neurology, regeneration, and illness, but they have been underutilized in orthopaedics[1]. Our research has shown in an *in-vitro* model that the corrosion of implants and the release of metal ions and particles can result in early implant failure[2], increasing the risk of toxicity to neighboring tissues. Both CoCrMo particles and titanium ions have been used to study these phenomena. The significance of vitamin E's antioxidant capabilities in reducing metal toxicity was also examined and our research showed that vitamin E significantly shields MG-63 cells against CoCrMo particles. Additionally, it slows down corrosion, coats implants in protection, and decreases particle-induced cellular toxicity. To further explore this subject, we used a zebrafish model to examine how metal particles, namely CrCoMo, affect embryonic development and the role of vitamin E's antioxidant potential.

METHODS: The study employed two distinct breeds of zebrafish: the wild type Ekkwill strain and the transgenic lyz:dsRed strain. The fertilized eggs were gathered and cultivated in a standard egg water solution comprising instant salt and methylene blue. A 1 mg/mL stock solution of the materials was originally made, followed by serial dilutions to obtain concentrations of 100 parts per million (ppm) for CoCr particles and 2.5 ppm for all antioxidants. These dilutions were carried out using molecular-grade water containing 5% phenol red and were intended for microinjections. During the 2-8 cell stage following fertilization, a volume of 4 nanoliters (nL) from each sample was microinjected into the yolk. The study aimed to assess the impact of injection on embryonic development and cardiovascular health by measuring the survival rate of embryos and their heartbeat rate at 24 and 48 hours post-injection. To assess the depletion of hair cells in the lateral line of zebrafish, a technique using DAPI staining was employed on viable embryos to specifically label the neuromasts. The embryos at 3 days post fertilization (dpf) were subjected to dechlorination and were exposed to a 1:1000 dilution of DAPI (1µg/mL) solution in 6 well plates. Following a 2-hour incubation period, the embryos were seen using the fluorescent microscope (EVOS M5000). To investigate inflammatory responses and visualize neutrophil activities affected by CoCr particles, we utilized the Tg(lyz:dsRed), a transgenic zebrafish model that genetically labels neutrophils with red fluorescence protein. A total volume of 4 nL containing CoCr wear particles at concentrations of 100, 500, and 1000 ppm, respectively, were microinjected in the yolk area of zebrafish embryos at 3 dpf. A control group received an injection of ordinary blue water.

RESULTS: Based on our analysis of embryo survival and heartbeat rates, it was observed that 50% of the embryos exhibited viability when exposed to a concentration of 100 ppm of CoCr wear particles. However, when antioxidants were introduced alongside wear particles, the survival rates significantly improved. Specifically, the addition of vitamin E resulted in an 85% survival rate, carvacrol led to an 80% survival rate, and eugenol yielded a 90% survival rate. Despite the presence of low survival rates and the occurrence of ill embryos, the observed variations in the heartbeat rate of the embryos were not found to be statistically significant. The lateral line of zebrafish has distinct advantages that render it a valuable tool for studying *in vivo* toxicity. A total of 12 neuromasts were seen on the lateral line of the control embryos, but the embryos treated with CoCr wear particles at a concentration of 100 ppm exhibited only 4 neuromasts on their lateral line. The embryos subjected to wear particles in the presence of antioxidants exhibited a range of 6 to 8 neuromasts along their lateral line. Our investigation into the neutrophil reaction showed that an escalation in the concentration of wear particles corresponded to a concurrent augmentation in the neutrophil response. This finding substantiates the notion that wear particles had the capacity to induce inflammatory effects on the embryo.



DISCUSSION: The present study aimed to assess the potential toxicological hazards linked to the wear particles of CoCr. We showed that exposure of embryos to CoCr particles decreased the survival rate, while the use of antioxidants resulted in a notable enhancement in the survival rate. No statistically significant alteration in the heart rate was found. The zebrafish possesses a sensory system known as the lateral line, which is comprised of clusters of hair cells referred to as neuromasts. These cells have a high susceptibility to toxicity. The findings of our investigation indicate that the wear and tear particles of CoCr exhibit toxicity towards zebrafish. Additionally, the antioxidant properties have the potential to effectively combat the toxicity from wear particles. In implants, such as those used in the hip, particles are released due to wear and tear, which can have detremential effects within the human body. Further investigation is necessary to elucidate the precise mechanism by which antioxidants exert their mitigating effects on the toxicity of particles in the zebrafish model.

SIGNIFICANCE: Our investigation successfully discerned that CoCr wear particles cause toxicity in zebrafish, a phenomenon that is directly proportional to the concentration of these particles. A comprehensive *in vivo* investigation is necessary to understand the underlying mechanism by which wear particles induce inflammatory responses and elucidate the potential of antioxidants in mitigating the toxicity associated with these wear particles.

Figure 1 (a) The survival rates of the embryos **(b)** The heartbeat rates of the embryos **(c)** Decrease in the neuromasts in the lateral line of the embryos treated with CoCr wear particles at 100 ppm concentration when compared the control group (d) A pictorial representation of developing zebrafish embryo and the dead embryo treated with particles.

REFERENCES: [1] Busse, B *et al.* (2020) [2] Manjunath, V *et al.* (2021)

ACKNOWLEDGEMENTS: The Blazer Foundation, NIH, UICOMR.