In Vivo Study of Biocompatibility and Toxicity of TiO_2 Nanoparticles in Early Development of Zebrafish (*Danio rerio)* Embryos

Hailey Davis, Destiny Branchen, and Kerry J. Lee
Biological Sciences Department, Florida Gulf Coast University, Fort Myers, FL 33965

ABSTRACT

Nanoparticles are a common ingredient contained in many beauty and hygiene products and are characterized by their nanoscopic size of 0-100 nanometers. These nanoparticles are so small they are capable of diffusing into cells of any living organism and have the potential to cause damaging effects such as deformities, DNA damage, or death. To investigate the affects of nanoparticles on the development of a living organism, zebrafish (*Danio rerio)* embryos were exposed to a solution containing various concentrations of titanium dioxide, TiO_2, nanoparticles from a commonly used liquid skin foundation and incubated for up to 120 hours. Every 24 hours, embryos were imaged and characterized in real-time using a bright field optical inverted microscope (BFOIM) equipped with a CCD camera. This helped us to visualize toxic affects and to determine the biocompatibility and toxicity of TiO_2 on embryo development, specifically whether exposure to TiO_2 was dosage-dependent. Fully developed zebrafish larvae were then preserved in formalin and prepared for a histological protocol. The results produced by the histology report will help determine correlation of deformities to embryos and accumulations of TiO_2. The data generated from this study will help us to understand the effects nanoparticles have on aquatic organisms and their environment.

INTRODUCTION

Nanotechnology research is an innovative technology that has become a vital tool in the study of nanoparticles and their effects on the body components in *in vivo* organisms. A nanoparticle is defined as a particle whose dimensions do not exceed 100 nanometers, and must contain characteristics different from larger molecules of the same element[1]. The main particle that will be investigated in this study is titanium dioxide, apparent as TiO_2. This nanoparticle has an average size of 24nm and is utilized for pigments in paints, cosmetics, foods, plastics, and other similar materials, for its bright appearance[4]. In 2005, over half of the TiO_2 being synthesized could be found in many paints, coatings, glazes, and enamels, but the production of TiO_2 continues to increase with a growing use in cosmetics and toothpaste[2]. Due to the infinitesimal size, these nanoparticles are able to penetrate, diffuse across selectively impermeable membranes, and embed themselves into any cell. The increasing number of nanoparticles today, and specifically TiO_2, has the potential to cause harmful and damaging effects to any organism that is exposed to a detrimental concentration of nanoparticles. To study the toxicity and biocompatibility of nanoparticles the Zebrafish (*Danio rerio*), is a common model organism. Zebrafish are beneficial for nanoparticle research due to unique characteristics they possess, such as a 120-hour complete development period, which is independent of parental organisms[3], a high fecundity rate, and maturation within transparent embryos, favorable for microscopic visualization. Studies have been conducted using silver (Ag) nanoparticles to track diffusion and effects on Zebrafish development. Results have shown that Ag nanoparticles diffused passively into membranes, and at higher concentrations, organism's experienced death and deformities, while lower concentrations allowed for normal development[4]. No investigations have been done on the effects of TiO_2 on an *in vivo* model.

METHODS

- To extract TiO_2 nanoparticles from a commonly used liquid foundation, a small amount of foundation was weighed out and transferred to a 50ml Eppendorf tube, combined with DI water, vortexed until completely mixed, and then incubated for 24 hours to allow separation of lipid and aqueous components. The aqueous layer, containing TiO_2, was characterized using DFOM[3].
- Breeding was performed between two male and three female zebrafish. Embryos were collected and washed twice with Egg water, then placed in a 24-well plate, with four embryos per well. Varying concentrations of TiO_2, including lower dosages of 0, 0.1, 0.2, 0.4, 0.6, 0.8 g/L and higher dosages of 0, 2, 4, 6, 8, 10 g/L were added to corresponding wells. Embryos were observed and imaged through a CCD camera and LFOIM at 24 hour intervals for 120 hours[2].
- Three trials were completed for each concentration of TiO_2 to collect adequate data and yield results.

RESULTS

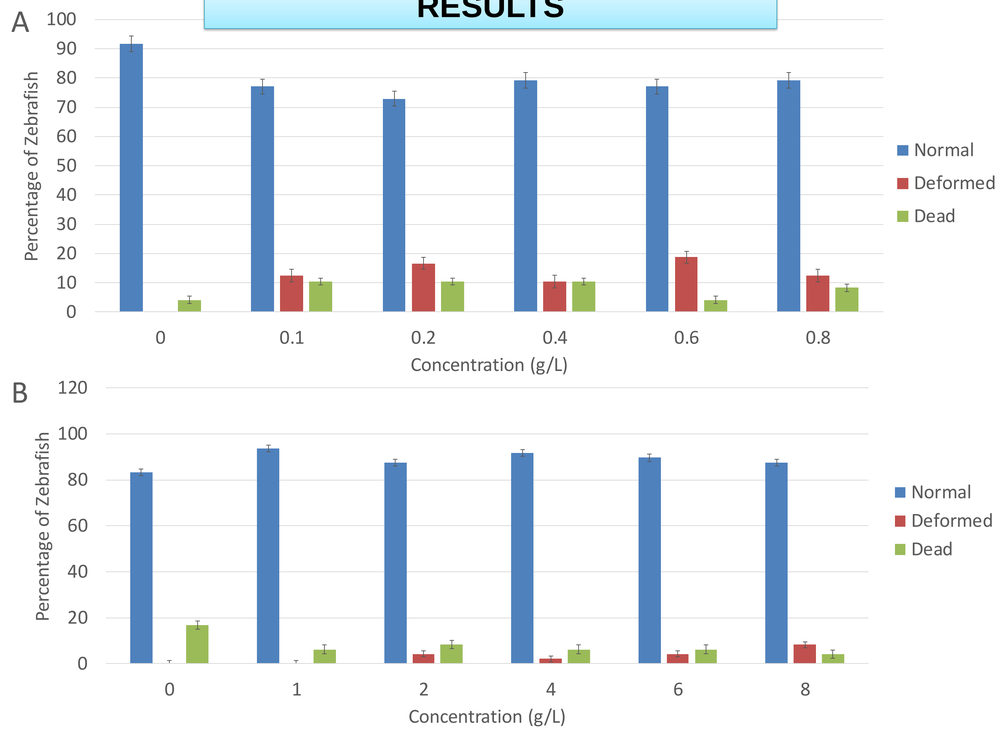

Figure 1: The percentage of normal, deformed, and dead zebrafish that were observed after 120 hours of exposure to various concentrations of TiO_2. A) Percentage of normal, deformed, and dead zebrafish at low concentrations of TiO_2. B) Percentage of normal, deformed, and dead zebrafish at high concentrations of TiO_2. T-test results indicated that TiO_2 was biocompatible in zebrafish embryo development.

Figure 2: The images in the top row display normal development of zebrafish at times 0hr, 24hrs, 48hrs, 72hrs, 96hrs, and 120hrs, respectively.
The images presented in the bottom row display abnormal development of zebrafish due to toxic dosages of TiO_2 at times 0hr, 24hrs, 48hrs, 72hrs, 96hrs, 120hrs, respectively.

CONCLUSION

To conclude, the control groups were compared to each treatment dosage of TiO_2 nanoparticles. The conducted student T-test revealed that all tested dosages of TiO_2 are biocompatible in zebrafish (Fig. 1 A, B); however, results indicate that low dosages of TiO_2 had more detrimental effects to the development of zebrafish embryos (Fig. 1 A), showing more deaths and deformities than seen in the high concentrations of TiO_2 (Fig. 2). This may be due to the possibility that at higher concentrations, the NPs aggregate or clump together and are not able to enter the developing embryo.
The results produced in conclusion of this experiment provide great insight for researchers due to the increased use of nanoparticles in various products today, and the damaging effects observed in many zebrafish at completion of our experiment. Although the results of our experiment did not produce significant findings, studies have been done in the past presenting results that reveal significant toxicity in various dosages of other nanoparticles. For example, a study conducted in 2015 using zinc oxide (ZnO) nanoparticle from a commonly used sunscreen, found that high dosages including 4,6, 8, and 10 g/L of ZnO were toxic to zebrafish development[5].

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