# The Use of a Designer Nanozyme to Mitigate Osteoclastic Formation

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**Introduction.** When in microgravity, astronauts lose bone rapidly and the disuse osteoporosis seen in astronauts shares many similarities to the degenerative osteoporosis displayed in normogravity (1g) for which there is no satisfactory treatment. Along with the lack of weight bearing in microgravity being considered a risk factor for bone loss, astronauts are also exposed to cosmic radiation and other environmental stress factors. At reactive surface sites, cerium ions have the ability to easily undergo redox cycling: drastically adjusting their electronic configurations and versatile catalytic activities. These properties make cerium oxide nanomaterials fascinating. We have previously shown that an engineered artificial nanozyme composed of cerium oxide nanoparticles (CeONPs) and designed to possess a higher fraction of trivalent ($Ce^{3+}$) surface sites, mitigates the ionizing radiation (IR)-induced loss in bone area, bone architecture, and strength in a rodent model. However, combined exposure to both microgravity and radiation has been shown to synergistically accelerate further bone loss in animal studies. Here, the aim of this study was to investigate the effects of this designer nanotherapeutic on the maturation of macrophage cells to mature osteoclasts under the combined effects of both microgravity and gamma radiation *in vitro*.

**Methods.** Cerium (III) nitrate hexahydrate was dissolved in deionized water and oxidized through addition of 3% hydrogen peroxide to a pH below 3.5. Particles were then stored away from light at room temperature for up to 30 days to allow for the surface catalyzed degradation of excess peroxide species and equilibration. Nanoparticle morphology, hydrodynamic radius, surface charge and composition were characterized using high-resolution transmission electron microscopy (HRTEM), a Zetasizer and x-ray photoelectron spectroscopy (XPS) respectively. RAW 264.7 macrophage cells were cultured in the NASA-developed rotating wall vessels (20 rpm for 48 hr) at the NASA Johnson Space Center to simulate microgravity (0g) on the ground. Cells were suspended in alpha-minimal essential medium (α-MEM Gibco, Grand Island, NY, USA) with 10% heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA), 100-U/mL penicillin, and 100-µg/mL streptomycin. Cells under simulated microgravity were subsequently exposed to 0.5 Gy of gamma radiation and then supplemented with CeONPs at a concentration of 10 µg/mL. Cell viability, $TRAP^+$ staining, and Elongation Index, were used to assess cell viability/proliferation/cytotoxicity, osteoclastic differentiation, and a M1 or M2 phenotype respectively. Statistical analysis was carried out using GraphPad Prism (version 8.0, US) and groups compared using the nonparametric Mann-Whitney test. $p$ values < 0.05 were considered significant.

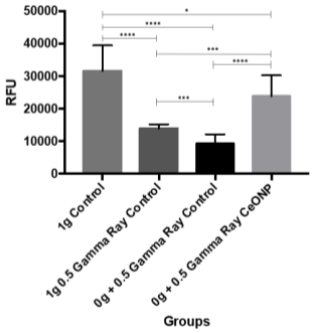


**Figure 1.** Macrophage metabolic activity. CeONPs protect against radiation- and simulated microgravity-induced dysfunction.

**Results.** Spherical particles composed of 60% $Ce^{3+}$ with a hydrodynamic size of ~35 nm and surface charge of 25.4 mV were created. Data analysis revealed cell exposure to 0g + 0.5 Gy significantly reduced macrophage metabolic activity when compared with the 1g + 0.5 Gy control ($p < 0.001$) and 1g control groups ($p < 0.0001$) (Figure 1). However, supplementation of macrophages with CeONPs when under 0g + 0.5 Gy conditions, significantly increased metabolic activity when compared with both the 0g + 0.5 Gy ($p < 0.0001$), and 1g + 0.5 Gy ($p < 0.001$) groups. Thus, suggesting CeONPs have the potential to rescue healthy cells despite the combined exposure to simulated microgravity and radiation. Further, CeONPs significantly decreased the incidence of multinucleated giant cells ($p < 0.05$), and $TRAP^+$ cells ($p < 0.0001$, Figure 2) when compared to 0g + 0.5 Gy exposed macrophages suggesting CeONPs potential in restricting osteoclast cell differentiation and potentially prolonging chronic inflammation. Finally, supplementation of macrophages with CeONPs resulted in significantly elongated, and spindle-like shaped cells, which are classically associated with the reparative M2 phenotype, despite exposure to both simulated microgravity and gamma irradiation (Figure 3).

**Discussion.** Our results reveal CeONPs may be a promising therapeutic agent against the inflammatory-induced space environment. As such, further work is warranted and future analysis will include but not be limited to morphological testing, RNA sequencing analysis, protein analysis, and flow cytometry analysis.

**Significance.** Extreme bone loss within the space environment remains an important challenge to solve. Presently, no available efficacious approaches definitively prevent and treat this bone loss. Our results suggest that CeONPs hold promise as a potential therapeutic agent however, further work is needed to investigate their effect *in vivo*.

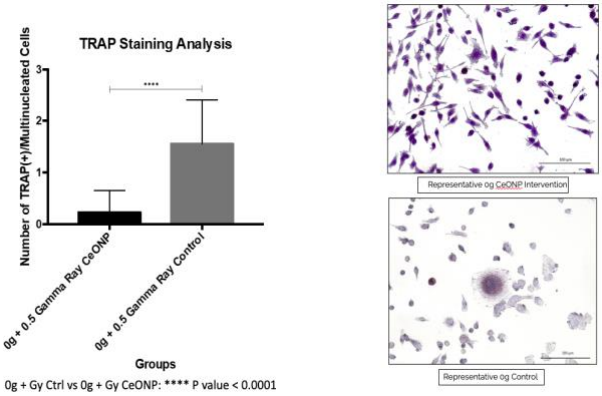


**Figure 2.** $TRAP^+$ staining. Data show CeONPs protect against radiation- and simulated microgravity-induced osteoclastic differentiation and activation *in vitro*.

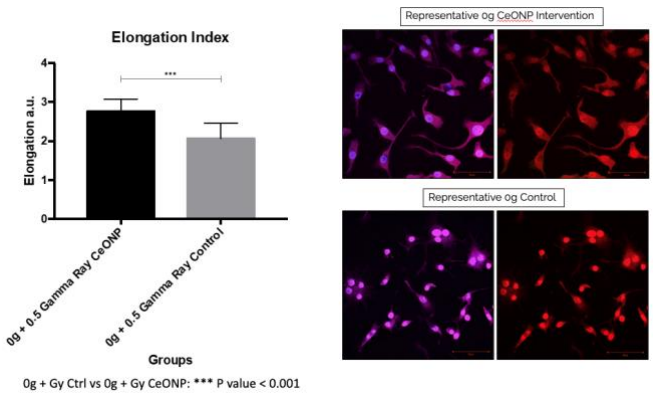


**Figure 3.** Despite exposure to radiation- and simulated microgravity-induced culture conditions, following supplementation with CeONPs, the macrophages display a M2 phenotype as evidenced by a significantly increased elongation index.