A New Method to Assess Cytotoxicity of Osteoblasts Exposed to EDTA

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Birth defect screenings are traditionally performed on live animals, exposing pregnant mothers to harmful toxicants. However, this method is ethically problematic and requires the sacrifice of many animals. To address this issue, researchers are exploring alternative screening methods. Embryonic stem cells (ESCs) provide a promising alternative because they can differentiate into different cell types and allow researchers to study the effects of different chemicals on organism development without harming animals. Our lab has developed an in vitro osteogenic protocol using ESCs that allows for the differentiation of ESCs into osteoblasts while exposing the cells to different chemicals to assess developmental toxicity on the skeleton. While the tetrazolium-based colorimetric assay (MTT assay) is commonly used to measure cytotoxicity, it can be time-consuming and requires expensive reagents and equipment. Therefore, there is an increasing interest in developing alternative, more cost-effective, and simpler methods that can achieve similar results. To address this need, we have developed an alternative methodology that utilizes Hematoxylin staining to count cells and evaluate cell toxicity. However, osteoblast differentiation involves calcification, which can interfere with the staining process. Therefore, we developed a methodology that includes treating cells with EDTA to remove calcification without damaging the cells, followed by Hematoxylin staining of the nuclei to count cells. This new methodology provides a more efficient and ethical way to screen for developmental toxicity in bone structure.