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A Novel Method to Manipulate Osteoblastic Differentiation

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Osteoporosis is the thinning of bone tissue and loss of bone density over time, leading to an increased risk of fracture. This prevalent bone disease results when the bone, a living tissue, fails to form sufficient new bone, while existing bone is reabsorbed by the body at a greater rate. Since mammals have limited regenerative ability, the production of new bone as a result of this disease is extremely problematic. Of interest in developing a cure for osteoporosis, is the ability to manipulate osteoblast differentiation. Osteoblasts are specialized mesenchymal cells that are responsible for bone formation. The remodeling and maintenance of bone tissue relies on the balance between bone resorption and bone formation; osteoblasts are responsible for the formation of new bone, while osteoclasts are responsible for resorption of bone [1].

Osteoblastic differentiation *in vivo* is a three step process, including cell proliferation, matrix maturation, and matrix mineralization. Since osteoblasts are responsible for bone formation, both the proliferation and mineralization phases of differentiation are essential to further strengthening bone in patients with osteoporosis [2]. Dedifferentiation is defined as a cellular process in which a partially or completely differentiated cell reverts to an earlier developmental stage. Mammals have a very limited ability to regenerate bone, however, other vertebrates, such as zebrafish, have the ability to entirely restore amputated bony structures through dedifferentiation of the mature osteoblasts. The goal of this *in vitro* study was to determine the effect of the chelator, zeolite, on osteoblastic differentiation, and develop treatment methods for osteoporosis by means of osteogenesis and regenerative bone tissue [3].

Zeolite is a microporous, aluminosilicate mineral used for its ability to increase DNA synthesis in normal osteoblasts [4]. In this study, osteoblasts were treated with varying concentrations of zeolite from 0.1 μg/mL to 100 μg/mL. Osteoblast production was stimulated by zeolite which correlates with increased proliferation. Alkaline phosphatase (ALP) staining was used to determine the effect of zeolite on the osteoblast differentiation process. ALP is an enzymatic marker of bone formation in the mineralization phase, thus a decrease in ALP as the concentration of zeolite increase indicates dedifferentiation. In order to confirm these results, ELISAs were performed to measure proteins found specifically during the proliferation and mineralization phases: TGF-beta and osteopontin, respectively. ELISAs showed a threshold concentration, 0.1 μg/mL, above which dedifferentiation is stimulated, returning osteoblasts to the proliferative stage.

After treating the osteoblasts with zeolite, the cells were imaged to determine the ability of zeolite to pass through the plasma membrane into the cell, and if any morphological changes were induced by the treatment. In the control images, there were far fewer free floating ribosomes found in the cells and the endoplasmic reticulum was depicted very clearly. In the experimental images, the endoplasmic reticulum was substantially diffuse and there were notably more free ribosomes in the cytosol. In conclusion, this extrinsic response that prompts dedifferentiation of osteoblasts is believed to have similar regenerative ability to that of the bony fin structures occurring in zebrafish.

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[5] The authors would like to acknowledge the administration of the Bergen County Technical Schools for their continued support of the research program.

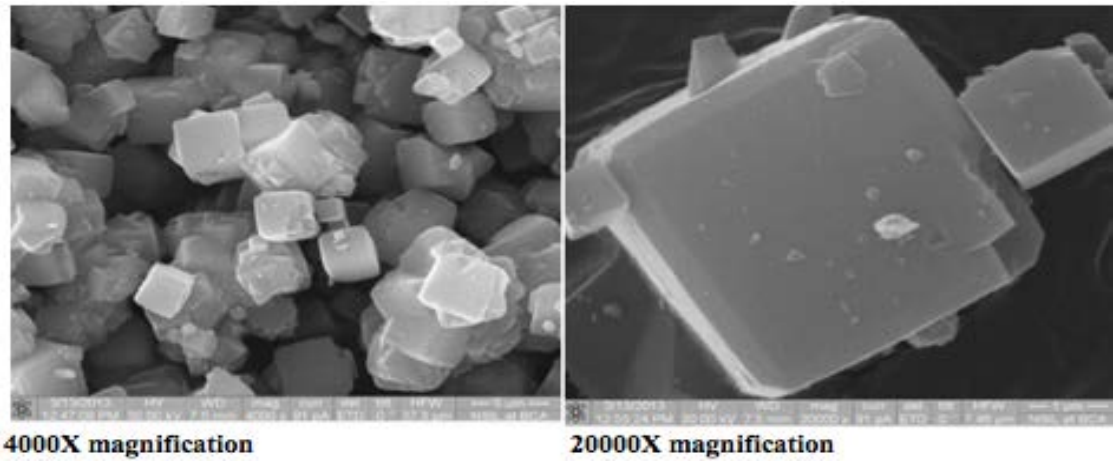

Figure 1. SEM micrographs of zeolite particles to analyze structure. Zeolite showed a cuboidal shape and measured approximately 2.0 μm in length.

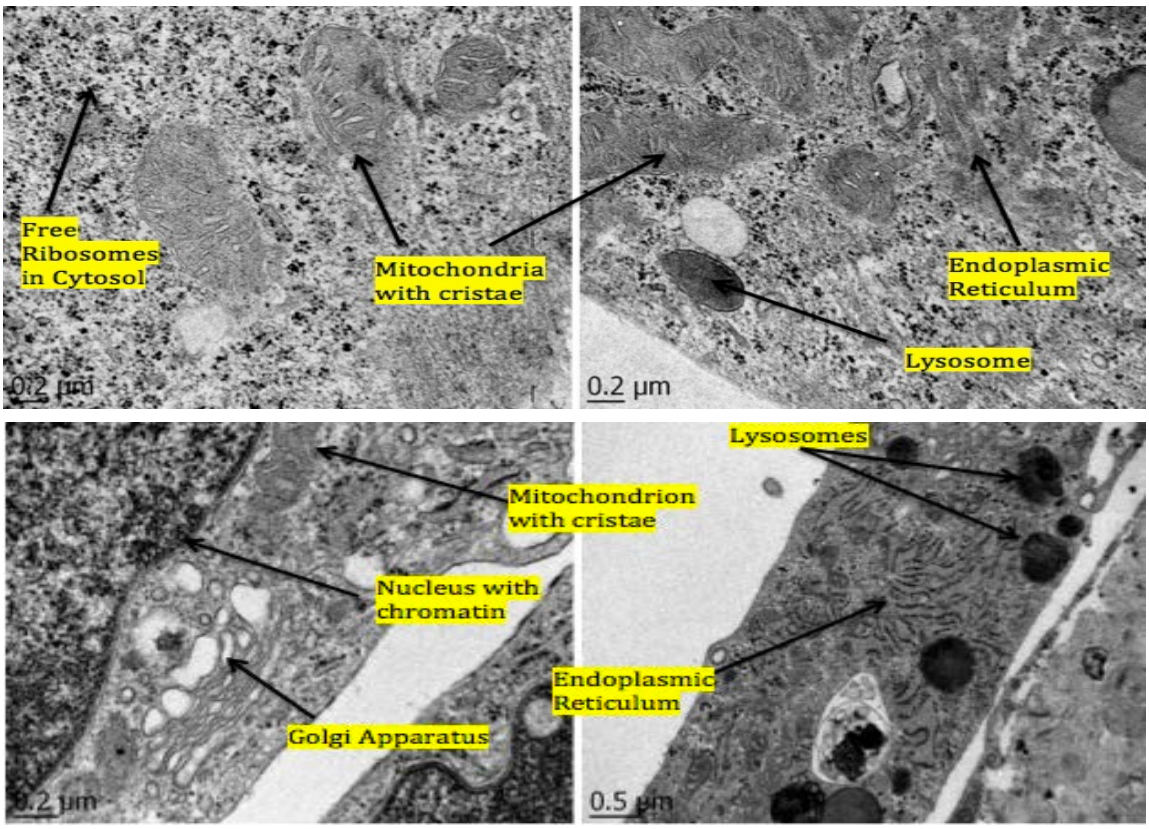

Figure 2. TEM micrographs of osteoblasts to determine absorption of zeolite. Top: control, untreated osteoblasts. Bottom: osteoblasts treated with zeolite; endoplasmic reticulum and ribosomes are more diffuse throughout the cell. The ribosomes are not clearly bound to the ER as in the control cells.

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