**The Use of Earth Metals to Stimulate Bone Growth**

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**INTRODUCTION:** Strontium (Sr) and Niobium (Nb) are two elements that have shown therapeutic potential in bone trauma recovery and degenerative bone diseases like osteoporosis. Sr is thought to have both anabolic and anti-resorptive effects through inducing the proliferation of osteoclasts as well as inhibiting the proliferation of osteoclasts.[1] On the other hand, Nb was found to increase alkaline phosphatase activity (ALP) and calcium deposits, improving cell proliferation as well as aiding in osseointegration of implants and scaffolds. However, the literature had varying results on optimal concentrations to be used. The purpose of this study is to further explore the cytotoxicity of both elements on osteoblasts and to find the effective doses of both Sr and Nb for use in situ to treat bone healing and degenerative bone diseases. We hypothesize that increasing doses of these elements will increase osteoblasts’ proliferation up to a cytotoxic point.

**METHODS:** Human osteosarcoma cell line (Saos-2) were treated with Nb concentrations of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, and 5 mM; or Sr at 0.25, 0.5, 1, 5, 10, 25, 50, 100 mM; or a mixture of Nb of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1 mM while holding Sr constant at 25 mM. The cells were maintained as monolayer cultures in a humidified incubator at 37°C and 5% CO2. OptiMEM media containing 10% FBS and 1% penicillin/streptomycin was exchanged every other day and the cells were cultured for 2 weeks. AlamarBlue (Resazurin) assay is a redox indicator that was used to evaluate metabolic activity and cellular health.[2] ALP activity was used as a biochemical marker for osteoblast proliferation indicating early bone growth. ALP activity was measured using p-nitrophenyl phosphate (pNPP) as a phosphatase substrate which turns yellow when dephosphorylated by ALP and calculated as follows:[3]:

\[ \text{ALP activity (U/ml)} = \frac{A}{V} \]

Where A is amount of pNPP generated (in µmol), V is volume of sample (in ml). Alizarin red staining (ARS) was used to stain calcium deposits and bone nodules formation at 28 days post-treatment. Statistical analyses were performed with Prism 7 (GraphPad Software Inc).

**RESULTS:**

**Cells treated with Nb only:**

Nb had no effect on proliferation rate when compared to control after 3 days (Panel 1A). However, after 5 days, cells treated with Nb concentrations over 0.1mM showed decreased proliferation while 0.025 mM and 0.05 mM of Nb showed higher proliferation rates than control at the end of the 14 days. Per panel 2A, as the concentration of Nb increases, there is a slight increase in ALP activity, but it is only significantly different at 0.1 mM after 10 days. Increasing Nb concentrations more than 0.1 mM caused a significant decrease in activity especially after 7 days. Interestingly, ALP activity for the same concentrations displayed a bull curve with activity peaking at day 5 (except in 5 mM).

**Cells Treated with Sr:**

All concentrations of Sr, including control (Panel 1B), displayed a bimodal distribution with the first peak after 5 days in concentrations < 1mM or 3 days in concentrations > 1mM and at 10 days except for 1 mM which showed the highest proliferation overall and a linear increase in proliferation without plateauing after 10 days. Per panel 2B, low concentrations showed little to no change in ALP activity. At concentrations 1mM and 5mM, ALP had the highest activity after 10 days. At concentrations of 25mM, ALP activity increased earlier and stabilized around 1.25 U/ml before decreasing at day 10. Increases in proliferation were generally followed by an increase in ALP activity.

**Cells treated with Nb and Sr:**

Panel 1C shows that all conditions showed a bimodal distribution with the control peaking a measurement point later than treated cells peaked. There is a slight increase in proliferation between low concentrations of Nb and the control up to 0.125 mM. Further increase in concentration is similar to control. It appears that increasing the concentration of Sr decreased proliferation slightly when mixed with Nb in the higher concentrations. Interestingly, when looking at panel 2C, all concentrations of Nb and Sr decreased ALP activity to baseline levels overtime. The control has nearly double the ALP activity after 3 days and almost triple after 10 days.

**DISCUSSION:** Based on cell proliferation, cells treated with Nb only and Sr only had the highest proliferation levels at 0.025 mM and 1 mM, respectively. Based on ALP activity, cells treated with Nb only and Sr only had the highest differentiation levels after 10 days at 0.1 mM and 1 mM, respectively. However, mixing Nb and Sr significantly decreased ALP activity while proliferation was unaffected. This could possibly indicate a synergic inhibitory effect on osteoblast proliferation which requires further investigation. Nb concentrations over 1 mM and Sr concentrations over 5 mM appear to be cytotoxic with decreases in ALP activity. However, 25 mM of Sr showed an increase in proliferation seen in days 1 through 7, which can possibly be used as a loading dose.

**SIGNIFICANCE/CLINICAL RELEVANCE:** The study of the combinatory effect of Sr and Nb on osteoblast proliferation and growth will increase the efficacy of their use as therapeutics for bone healing and degenerative bone diseases.