

Rodrigues A. I., Leonor I. B., Reis R. L., van Blitterswijk C., and Habibovic P., "Stimulatory effects of inorganic ions on osteogenesis in vitro", *Journal of Tissue Engineering and Regenerative Medicine*, 2014.

## Introduction

Several studies demonstrated the effect of silicate ions (Si) on differentiation of bone precursor cells<sup>1,2</sup>, although its exact role in processes related to bone formation and remodeling is still incompletely understood. The focus of this work is to explore the effect of calcium and silicate ions on growth and osteogenic differentiation of human mesenchymal stem cells (hMSCs). This strategy may reduce the need for growth factors required to stimulate bone formation in regenerative approaches, decreasing the associated costs and overcoming stability issues.

## Materials and Methods

In order to define the range of Si concentrations that are not toxic to cells, we performed a preliminary study varying Si concentrations from 0.00357mM to 4mM. The concentration of the Ca ions was selected based on the earlier study by Barradas et. al<sup>3</sup>. Cell culture media were supplemented by using sodium silicate ( $\text{Na}_2\text{SiO}_3$ ) and/or calcium chloride dehydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) as Si and Ca precursors, respectively. hMSCs derived from bone marrow were seeded at a seeding density of 2.000 cells/cm<sup>2</sup> and allowed to adhere overnight. Then, the medium was replaced by the appropriate supplemented medium and cells were cultured for 3, 7, 14 and 18 days. Basic and osteogenic media were used as negative and positive controls. Cell proliferation was evaluated by DNA quantification. hMSCs osteogenic gene expression was evaluated by Q-PCR.

## Results

DNA quantification indicated an increase in cell number during the culture time for all the conditions. Results obtained by Q-PCR revealed a significantly higher expression of osteocalcin (OC) and bone morphogenetic protein-2 (BMP2) in cells cultured in media supplemented by both ions, as compared to media containing either Ca or Si alone.

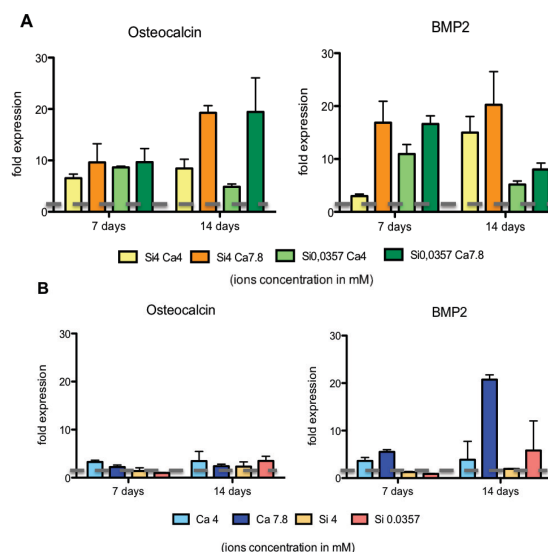


Fig. 1. Q-PCR results for OC and BMP-2 for the combination of ions (A) and for Ca and Si alone (B).

## Discussion and Conclusions

DNA quantification studies indicated that none of the selected concentrations had a negative influence on cell proliferation. The increase in osteogenic gene expression for cells cultured with both Ca and Si suggested a synergistic effect of the two ions on osteogenic differentiation of hMSCs. We further showed that cells cultured in the medium with the highest concentration of Ca (7.8mM) revealed a higher expression of the selected genes, which is in accordance with the earlier results by Barradas et al<sup>3</sup>. The obtained results suggest the importance of combining both ions, Ca and Si, for promoting the osteogenic differentiation of hMSCs.

## References

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## Disclosures

The authors have nothing to disclose.