Osteoblast Differentiation in SrO and MgO Doped Tricalcium Phosphate; Gene Expression Study

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Statement of Purpose: β-tricalcium phosphate (β-TCP) is widely used in bone grafting applications due to its promising biocompatibility and osteoconductivity. Trace element addition is proved to enhance osteoinductivity, osteogenesis and angiogenesis. Sr^{2+} induces osteogenesis by increasing osteoprotegerin (OPG) production. Mg^{2+} also enhances osteoblast cell attachment, proliferation and differentiation [1]. We have processed SrO and MgO doped β-TCP porous scaffolds using emulsion method. Our objective was to understand the effects of dopants on gene expression. Our hypothesis is that addition of these dopants will alter the gene expression by osteoblast cells which in turn would affect the bone remodeling process.

Methods: β-TCP was synthesized via solid state reaction between dicalcium phosphate and calcium carbonate. Doped powders were prepared by mixing β-TCP powder with 1 wt% MgO/SrO, followed by wet milling in ethanol. Samples were made by mixing dried TCP powders with appropriate amount of paraffin oil and emulsifier solution [2]. Formed slurries were transferred in disk mold followed by drying at 70 °C. Final sintering was performed at 1250 °C. Osteoblast cells (OBs) were seeded on samples and culture was performed at 34 °C. RT-PCR was performed using bone morphogenic protein receptor-2 (BMP-2), runt related transcription factor 2 (Runx2), receptor activator of nuclear factor kappa-B ligand (RANKL) and OPG primers. Field emission secondary electron microscopy (FESEM) was used to study the cellular morphology and its evolution.

Results: XRD results showed that MgO and SrO retards β-TCP to α-TCP transformation [1&3]. Figure 1a shows the RT-PCR data after 21 days of study. Sr^{2+} addition enhanced OPG and BMP-2 expression almost three times, and RANKL and Runx2 production two times than pure TCP. Mg^{2+}, on the other hand, did not have significant effect on BMP-2 expression compared to pure TCP samples. OPG and Runx2 expression was significantly increase by Mg^{2+} doping. These results are comparable to data related to day 28 of the study (figure 1b). Presence of Sr^{2+} accelerated OPG expression, whereas BMP-2 and RANKL production decreased, compared to day 21 but still higher than pure sample. OPG, production was increased in Mg-doped TCP after 28 days; however, a reduction in BMP-2, RANKL, and Runx2 expression was found. Significant BMP-2 production in SrO doped-TCP samples indicates the ongoing cellular differentiation at day 21. Decrease in BMP-2 expression as the OB differentiation factor from day 21 to day 28 in all samples reveals that samples had gone through cell differentiation. Runx2 activity on doped samples was upregulated at both time points. SEM images show that cellular monolayer was formed at day 21 and differentiation was started in doped samples. These results are aligned with those found by BMP-2 expression. Highest OPG:RANKL in MgO doped-TCP samples indicates that Mg^{2+} addition delays the osteoclastogenesis at day 21. All samples showed the increase in OPG:RANKL at day 28 compared to day 21. Pure sample had the highest increase from ~0.75 to ~2.3. This proves the decrease in remodeling and prolonged bone growth. SEM images show the bone lining cells with flattened structure on doped samples at day 28 (figure 2).

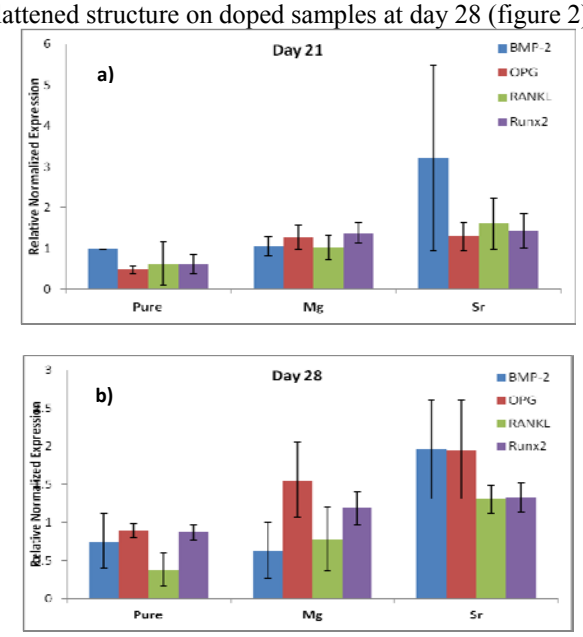

Figure 1. Normalized gene expression after 21 and 28 days.

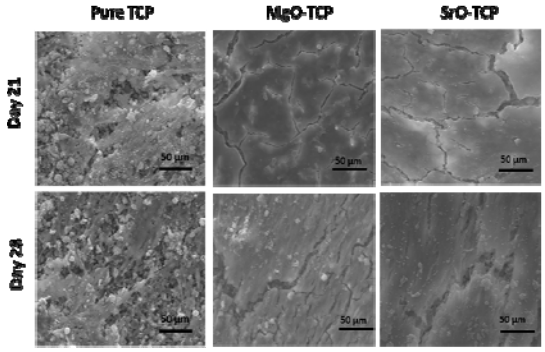

Figure 2. SEM micrograph of OB morphology after 21 and 28 days.

Conclusion: Addition of SrO and MgO to TCP porous scaffolds had significant effect on osteoblast gene expression. Osteoclastogenesis was hindered on MgO doped-TCP, compared to other two samples at day 21. At day 28, all samples had gone through cellular differentiation. In addition, bone remodeling decreased in all three compositions at this time point.

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References:

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