In vitro Characterization of Lithium-Doped Tricalcium Phosphate for Bone Graft

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Statement of Purpose: β-tricalcium phosphate (TCP) is a bioresorbable ceramic that has been widely studied for applications in orthopedics and tissue engineering. There has been much interest in the area of metal-ion substituted TCP, due to its improved mechanical and biological properties, which shows great promise for bone-tissue engineering applications [1]. Lithium is an element known to mimic the Wnt signaling pathway, which plays a central role in osteoblast proliferation and differentiation [2]. The **objective** of this study is to investigate the influence of Li-doping on the mechanical and biological properties of both synthesized and commercial β-tricalcium phosphate ceramics. Our **hypothesis** is that Li-dopants will induce an increase in osteoblast proliferative activity and differentiation when cultured on TCP. The **rationale** is that once we understand the role of dopants on cell-material interaction, we should be able to determine the optimal composition of Li in TCP for bone-tissue engineering.

Methods: Two groups consisting of three compositions each were investigated in this study. Group 1 consisted of pure synthesized β-TCP, β-TCP doped with 0.22 mol% LiCl, and β-TCP doped with 0.44 mol% LiCl, which were all synthesized using a wet coprecipitation method. The precipitate was dried, and the resulting powder was calcined at 800 ^{o}C. Group 2 consisted of pure commercial β-TCP, β-TCP doped with 0.25 wt% Li_2O, and β-TCP doped with 0.5 wt% Li_2O, which were all prepared by physically-mixing (PM). The powders for each sample composition were pressed into disc and cylindrical samples using a uniaxial press. The group of synthesized samples was sintered at 1120 ^{o}C, while the physically-mixed samples were sintered at 1250 ^{o}C. This difference in sintering temperature was necessary to retain pure β-phase. Phase analysis was performed using XRD, while chemical data was obtained by FTIR spectroscopy. The trend between Li-dopant and particle size, was investigated using a dynamic light scattering technique, while surface area was measured using BET analysis. *In vitro* cell interactions on all sample surfaces were used to investigate the culturing of human fetal osteoblast cells (hFOB) for 3, 7 and 11 days. An MTT assay was performed to determine the cell proliferation, and an ALP assay was performed to determine the level of cellular differentiation. Cell morphology on sample surfaces was evaluated with SEM.

Results: The average relative density of the synthesized samples were all above 95%, while the physically-mixed samples were lower, but still above 90%. XRD data showed that phase pure β-TCP was achieved in all samples. The surface area of the synthesized samples was 5.234m^2/g, 3.893 m^2/g and 2.078 m^2/g for the pure, 0.22 mol% and 0.44 mol%, respectively. While the synthesized samples showed a decrease in surface area with increasing Li-dopant concentration, there was no significant change in the physically-mixed samples. Particle size analysis supports the surface area findings, with no significant differences between the physically-mixed group, while particle size in the synthesized group shows a trend of increasing particle diameter as the amount of Li-dopant is increased. The MTT assay, as shown in **Figure 1**, indicate similar trends in both groups, with the lower concentration of Li-dopant showing increased proliferation compared to the pure sample, and the higher concentration indicating lower activity.

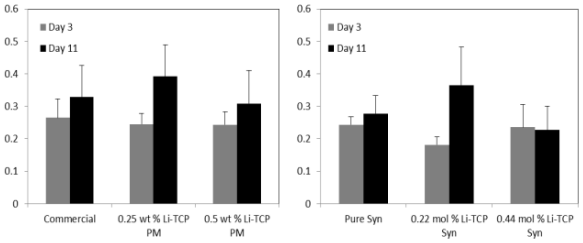

Figure 1. MTT assay of hFOB cells on pure and Li-doped b-TCP after 3 and 11 days incubation.

SEM morphologies of the hFOB cells, as shown in **Figure 2,** exhibited early stage cell adhesion, proliferation and mineralization on all of the Li-doped sample surfaces.

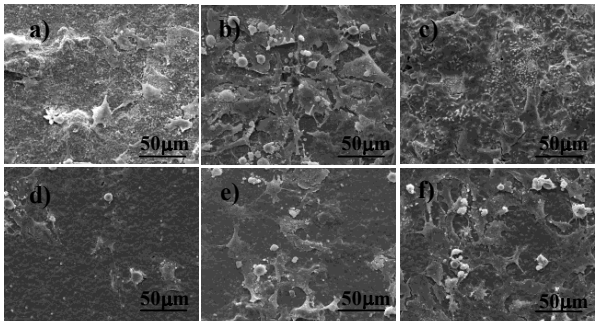

Figure 2. SEM micrographs presenting the hFOB cell morphologies on: a)Pure synthesized TCP,b) 0.22 % Li-TCP, c) 0.44 % Li-TCP, d) Commercial b-TCP, e) 0.25 % Li-TCP,f) 0.5 % Li-TCP

Conclusions: The addition of Lithium-based dopants to β-TCP does induce an effect on the cell-material interaction of osteoblast cells. Both of the groups considered in this study exhibited increased proliferative activity at the lower concentration of Li-doping, while the higher concentration showed a decrease in activity, indicating a toxic effect of Li at elevated doses, which is reported in literature. While this study shows the effect of Li-TCP on the proliferative activity of osteoblasts, work is being done to determine its influence on osteoblast differentiation.

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References:

[1] Banerjee, SS. Acta Bio. 2010;6:4167-4174.
[2] Spencer, GJ. J Cell Sci. 2006;119:1283-1296.