Polyphosphate dicalcium phosphate dehydrate (P-DCPD): A better strontium-eluting bone substitute (BS) for the bone defect repair

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Statement of Purpose: We recently developed an injectable polyphosphate dicalcium phosphate (P-DCPD) bone substitute (BS). P-DCPD is superior to existing calcium phosphate cements (CPCs) because of its mechanical strength, excellent anti-washout and controllable delivery of antibiotics, proteins and peptides in a sustained pattern. The aim of this study was to investigate the release profile of strontium (Sr), which has proven anabolic properties for bone, from P-DCPD and the influence of Sr loading on the cell growth and differentiation of murine osteoblastic MC3T3 cells.

Methods: P-DCPD was prepared by mixing calcium polyphosphate (CPP gel) with tetracalcium phosphate (TTCP) (1:0.87,wt/wt). For Sr-doping, Strontium carbonate (SrCO3) powder (Sigma) was mixed well with TTCP powder prior to adding CPP gel for setting (final Sr concentration was 10%, wt/wt, Sr-P). P-DCPD without Sr-doping was used as control (0%Sr-P). P-DCPD discs were submerged into deionized water in sealed containers and the volume maintained throughout the experiment in static conditions at 37 ºC. At the time points (2 h, 6 h, 1, 3,7, 14, 21, 28, 42, 56 and 70 days), eluents were collected and replaced with the same amount of deionized water. The collected eluents were stored at -20 ºC until use. The Sr release was measured by Inductively coupled plasma - optical emission spectrometry (ICP-OES). Collected Eluents were combined into three parts: early phase (2h-3d), middle phase (1-4w) and late phase (6-10w). MC3T3 cells were cultured in the presence of sterilized eluents (final 10%, v/v) for seven days. Cell growth was evaluated by MTT Assay. For cell differentiation, MC3T3 cells were cultured in osteogenic media containing and 10% eluent for14 days, and cell differentiation evaluated by alkaline phosphatase (AKP) assay.

Results: Sr release from 10% Sr-P up to 70 days was measured (see Fig. 1). Within the first 72 hrs, 10% Sr-P had significantly reduced Sr burst release. In addition, a sustained and zero-order Sr release was observed starting from day 3, up to 70 days. We found that the net concentration of Sr released at later phase (4.49±0.29 ppm, 6 to 10 wks) was significantly higher than that of early phase (1.71±0.07 ppm, 2 h to 3 d) and middle phase (2.02±1.43 ppm, 1 to 4 wks, $p<0.05$). As shown in Fig. 2, with known Sr concentration, eluents from 10% Sr-P were non-toxic to cells and slightly stimulated cell growth vs. eluent-free control (Fig. 2A). There was no significant difference of cell growth between 0% Sr-P and 10% Sr-P. A significantly increased AKP enzyme activity was observed in cells treated with eluents from 10% Sr-P compared to eluents from 0% Sr-P and the eluent-free control, particularly for the eluents collected at early and late phases (Fig. 2B, $p<0.05$).

Conclusion: Load-bearing bone defects remain a major clinical and socioeconomic problem. The synergy between P-DCPD and pharmacology has opened a wide field of possibilities, especially for the load-bearing bone defect healing. Data generated from this study was promising and may show the future application of Sr-P as a better drug-eluting ceramic BS for the full repair of load-bearing bone defects. Material optimization and validation in animal bone defect model are required moving forward for clinical application.

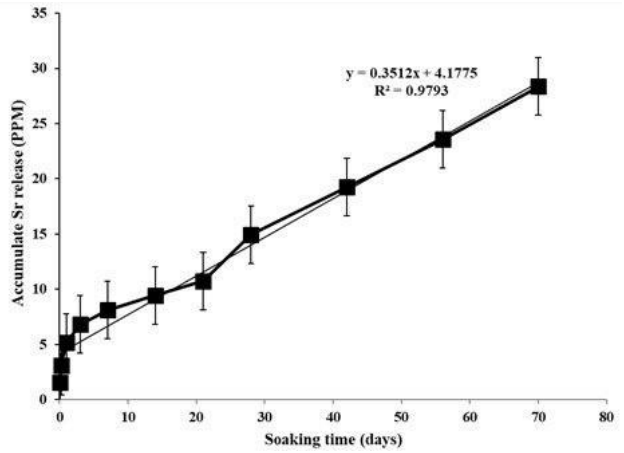

Fig. 1. Figure 1. Sr release from 10%Sr-P over 70 days (n = 3).

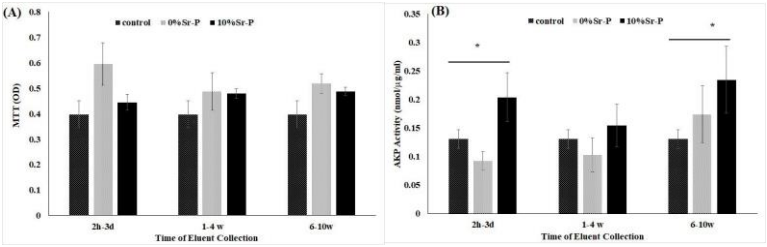

Fig. 2. Cell growth (MTT) of pre-osteoblastic MC3T3 cells grown on culture plate (control) when exposed to 10% eluents collected from 0% Sr-P and 10%Sr-P at different phases for 7 days (A, n=3); the normalized AKP activity (nmol/mg/ml) of MC3T3 cells for 14 days with the same eluent treatments (B, n-3). Cells cultured in the absence of eluent treatment was included as control. Statistical difference was indicated by (*) ($p < 0.05$).

References:

Ren W, et al. Setting mechanism of a new injectable Dicalcium Phosphate Dihydrate (DCPD) forming cement. Journal of the mechanical behavior of biomedical materials 2018;79:226-34