Synthesis and Characterization of Amorphous Magnesium Phosphate: A Novel Bone Cement Precursor

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Statement of Purpose: Calcium phosphate (CaP) based materials are widely explored and implemented for use in bone defect repair. Such materials are an excellent choice since the implant mimics the natural chemistry of mineralized bone matrix and in injectable cement form, can be implemented with relative ease. Of the available CaP cements, none fully meet the ideal standard, displaying low strengths and acidic setting reactions or slow setting times, and are non-resorbable[1]. More recently researchers have explored magnesium phosphate (MP) cements for clinical application. Although reports are limited, preliminary studies have shown that MP based cements may display higher strengths, more ideal reaction times and resorption rates than their CaP counterparts[2]. With the lack of knowledge on biomedical MP's, it is necessary to initiate developmental research from the ground level, most importantly understanding the chemical properties and biocompatibility of these cements and their precursors. In this study we synthesized and characterized an MP precursor, trimagnesium phosphate ($Mg_3(PO_4)_2$, TMP). Depending on the synthesis parameters, the TMP has the potential to be amorphous (ATMP) or crystalline (CTMP). While magnesium substitution has been shown to affect CaP crystallinity, to the best of the authors' knowledge this is the first presentation of the synthesis of amorphous magnesium phosphate and only the second study of biocompatibility of magnesium phosphate cement precursors[3,4].

Methods: ATMP and CTMP powders were previously synthesized through an aqueous precipitation reaction and characterized. Pellets of each (0.35grams, 13mm) were generated for subsequent *in vitro* testing, with pellets of β-tricalcium phosphate (βTCP) used as controls. MC-3T3 pre-osteoblasts were used for biocompatibility testing, grown in α-MEM media with 10% fetal bovine serum (FBS) and 1% antibiotics. Pellet solubility was assessed in *in vitro* culture conditions by intermittently changing the culture media and measuring Mg^{+} and Ca^{++} ions with ICP (ion coupled plasma spectroscopy). Biocompatibility was assessed with MTT cell activity assay, DNA quantification and cell fixation and dehydration for SEM imaging. Subsequently, pellets of ATMP were incubated for 2 weeks in α-MEM containing varying amounts of FBS (0% and 20%) to study surface changes.

Results: Previous unpublished synthesis results demonstrate that the aqueous approach yields amorphous magnesium phosphate powder which crystallizes upon heat treatment. ICP pellet solubility assessment of CTMP and ATMP showed that CTMP results in lower release of magnesium ions than ATMP over a 7 day period. Interestingly, ATMP shows a lower net calcium ion levels than baseline media, indicating that ATMP is somehow consuming calcium from media, more so than CTMP or β-TCP controls. MTT cell activity assay studies over 5 days of culture indicate comparable cell viability of ATMP to β-TCP but higher than CTMP. DNA quantification results, shown in Figure 1, indicate short-term growth is comparable between all pellets but at longer culture times ATMP and β-TCP perform better than CTMP. Figure 2 shows the changes in ATMP pellet surface with and without FBS presence. The 0% FBS pellet (a) shows significant changes from the native surface (c), likely due to the high solubility of the ATMP. The 20% FBS pellet (b) shows even more drastic changes. FBS appears to play a role in forming rosette patterns which appear beneficial to cell-surface interaction (d). Although characterization methods to date are inconclusive in identifying the altered composition, biomineralization principles and ICP results would indicate the rosette formulation comprise a Ca-Mg phosphate phase.

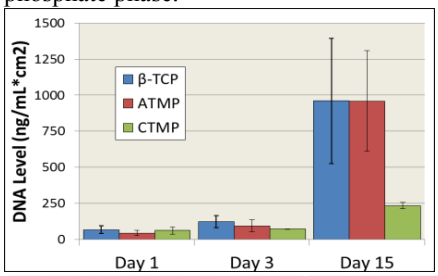

Fig 1: DNA level on pellets up to 15 days with ATMP comparable to BTCP and poor growth on CTMP.(n=3, figure shows avg+std dev)

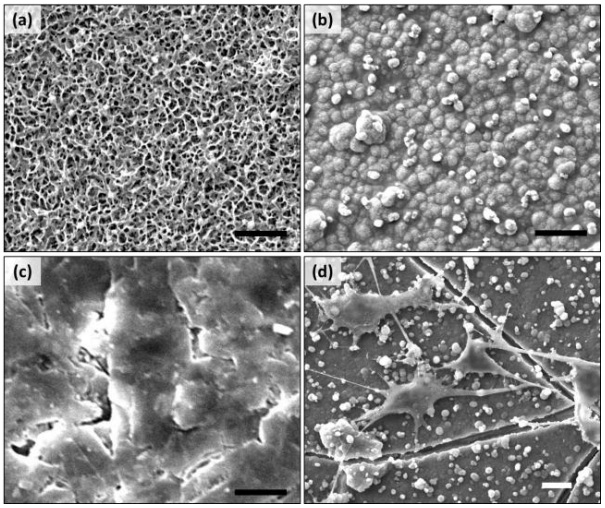

Fig. 2: Images represent ATMP soaked in media with 0% (a) and 20% (b) FBS , showing the surface changes from the virgin pellet surface (c). Scale: 5um. Image (d) shows MC-3T3 cell morphology on ATMP with rosette formation. Scale: 10um

Conclusions: Testing shows the ATMP to be non-toxic and potentially inducing biomineralization with osteoblast proliferation and viability levels similar to β-TCP controls. Future research will focus on in-depth studies of the biomineralization potential of ATMP and the utilization of ATMP as a precursor to create a clinically viable magnesium phosphate bone cements.

References: [1] Hofmann, M.P Acta Biomater. 2009; 5:43-49; [2] Yu, Y. Colloid Surface B. 2010; 76: 496-50; [3] Combes, C. Acta Biomater 2010; 6:3362-3378; [4] Tamimi F. Acta Biomater 2011; 7:2678-2865.