Amorphous Tri-Magnesium Phosphate (ATMP) as a Novel Bone Scaffolding Material

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Statement of Purpose: The utilization of calcium phosphate based materials has been widely explored and commercialized for bone repair and augmentation procedures. While these products are functional in some modality, no product to the best of our knowledge to date exhibits the ideal combination of desirable mechanical, physio-chemical and biological properties. More recently, magnesium phosphates have come into consideration as potential alternatives for bone augmentation materials. While the body of literature is substantially more limited, recent publications have found that various magnesium phosphate phases are non-toxic in vitro. In many ways, magnesium phosphate is being explored as a calcium phosphate analog. While amorphous calcium phosphate (ACP) has been explored in great depth for bone applications, and the use of magnesium substitution to stabilize amorphous calcium phosphate is known, amorphous magnesium phosphate (AMP) has only recently gained attention. In this work, the unique in vitro properties and cytocompatibility of an AMP synthesized by a new approach are explored.

Methods: ATMP and crystalline (CTMP) powders were derived via aqueous precipitation and characterized. Pellet scaffolds of each material were generated for subsequent *in vitro* testing, with β-tricalcium phosphate (βTCP) used as controls. Pellet solubility and stability was assessed under *in vitro* culture conditions by measuring Mg^{+} and Ca^{++} ions with ICP (ion coupled plasma spectroscopy) release into media and analyzing the changes in pellet surface through SEM, TEM and FTIR. Cytocompatibility with MC3T3 pre-osteoblast cells was assessed with MTT cell activity assay, as well as ALP expression and cell fixation and dehydration for SEM imaging. Cytocompatibility and polynucleation of with RAW 264.7 monocytes exposed to RANKL was assessed through staining of actin and nuclei, observing for osteoclast formation. Subsequently, further characterization of surface mineralization on ATMP pellets was carried out through SEM, TEM, XRD and FTIR while varying amounts of medium serum and length of incubation time.

Results: Analysis of synthesized magnesium phosphate, through XRD, SEM, TEM, FTIR, and TGA/DSC, demonstrate that the aqueous approach yields a highly stable amorphous magnesium phosphate powder which crystallizes upon high thermal treatment. Dissolution 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 consuming calcium from media. Direct MTT activity assay of MC3T3 cells indicate comparable cell viability of ATMP to β-TCP but higher than CTMP while indirect MTT shows the opposite trend, of higher viability on the CTMP than ATMP. The high magnesium ion concentration should yield lower cell viability on ATMP substrates, but it does not. This indicates a mediating factor in cellular viability. ATMP also displayed earlier increase in ALP expression than β-TCP or CTMP substrates, shown in Figure 1a. The additional factor was determined to be the spontaneous mineralization of the ATMP surface (not seen on the CTMP or β-TCP surfaces.) Interestingly, the study of osteoclast formation indicates that both the ATMP and CTMP substrates supported high levels of monocyte proliferation but suspended osteoclast formation, indicated by the lack of polynucleated cells observed. Imaging of ATMP surfaces indicated the spontaneous growth of an apatite-like rosette structure. This structure is the cause of the calcium depletion from culture media and appears to be the cause of improved MC3T3 viability on the ATMP substrates. Figure 1b shows the preferential orientation of MC3T3 cytoplasm attached to the apatite-like growths. Figure 2a shows the rosette growth on SEM. TEM and FTIR analysis of the rosette growth however indicate that the plate-like particles, while appearing in the form of *in vitro* formed hydroxaptite, are in fact amorphous, calcium-rich structures, seen in Figure 2b&c.

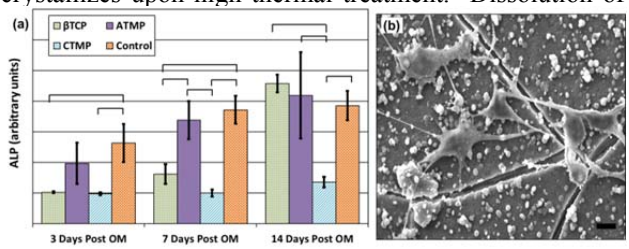

Fig 1 a) ALP expression on pellet surfaces as 3, 7 and 14 days and b) SEM of MC3T3 on ATMP surface (scale:10μm)

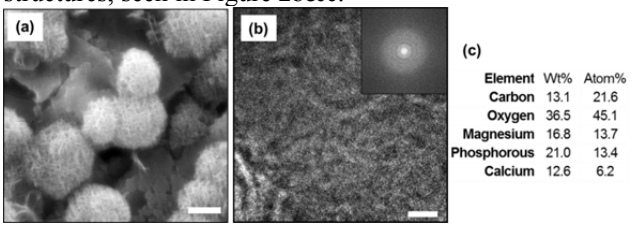

Fig. 2: a) Image of rosette growth on ATMP surface (scale: 1μm) b) TEM image and diffraction pattern of rosette growth (scale:5nm) and c) EDS elemental analysis of rosette growth.

Conclusions: Testing shows the ATMP to be non-toxic and inducing *in vitro* biomineralization with high osteoblast proliferation and ALP expression while suspending osteoclast formation. The surface mineralization formed on the amorphous magnesium phosphate is a calcium-rich, apatite structure which improves the osteoblast viability. Future research will focus on the biomineralization potential of ATMP with 3D porous structures and under *in vivo* conditions. Additionally, the utilization of ATMP as a precursor to create unique magnesium phosphate bone cements will be explored in the near future.

References: [1] Tamimi F. Acta Biomater 2011; 7:2678-2865. [2] Zhou, H. J. Mat Sci Mat Med 2012; 23: 2831-7