Effect of Mannitol Porogen Addition on Magnesium Phosphate Cements

Nicole J. Ostrowski[1], Bouen Lee[1], Abhijit Roy[1], Prashant N. Kumta[1]
[1]Department of Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Statement of Purpose: The study of magnesium phosphates for bone cements is a relatively new field compared to traditional calcium phosphate bone cements (CPCs). CPCs have been utilized because the composition closely mimics mineralized bone matrix. However, mechanical strength and resorption rate of these cements are not ideal [1]. Recent studies show that magnesium phosphates have higher strengths and faster resorption rates than the calcium counterparts [2-4]. Typical cement formulations comprise dead burnt MgO with a soluble phosphate salt. Porosity is widely regarded as a necessary parameter for the generation of functionally integrated scaffolds through the addition of salt or sugar crystals, the use of degradable fibers, 3D printing or the use of gases [5, 6]. Pore structure should be integrated to permit cellular infiltration and migration as well as mass transport for cellular nutrition. Particularly with ceramic structures, the pore structure negatively impacts the strength of the scaffold, and thus it is necessary to optimize the porosity and strength of the scaffold. The goal of this study is to evaluate the role of mannitol, a soluble pore former on the workability, mechanical durability and cytocompatibility of a magnesium ammonium phosphate cement system.

Methods: $Mg_3(PO_4)_2$ powder was synthesized through an aqueous precipitation reaction and thermal treatment (800°C, 6 hours) then lightly ground with 0, 10, 20, 30, 40, or 50 weight % mannitol sugar crystals. A 3M, pH 7.0 ammonium phosphate solution was used for the cement reaction, at various powder-to-liquid ratios (P:L). The 1st and 2nd setting times (ST) were determined using a Gillmore Needle Apparatus. Handling characteristics were qualitatively assessed based on consistency and injectability. Resulting cements were incubated in PBS at 37°C and characterized for presence of phase and molecular linkages (XRD and FTIR), surface area (SSA), porosity (true and apparent density) as well as pH and preliminary *in vitro* cytocompatibility with live/dead staining of MC3T3 cells.

Results: Pore former can change the properties of the cement in question and hence the first goal was focused on determining the most functional P:L for each amount of porogen. Specifically, P:L ratios which provide a 1st ST of 8-10 minutes and a 2nd ST 16-20 minutes at room temperature as well as a moldable to flowable putty consistency were chosen for further study. These STs have been described as most optimal for the majority of clinical and dental applications. STs are shown in **Figure 1**, where the optimal P:L have been denoted with blue/green bars. All further testing was completed on optimized embodiments. Cements were incubated in PBS for 0, 1, 3, 5, 7 and 14 days. True density measurements indicate at 0 days (no PBS incubation) the density incrementally decreased from 2.3g/cm^3 to 1.9g/cm^3 with increased mannitol content. Following incubation in PBS for greater than 24 hours, the true density for all samples was ~ 2.3g/cm3, indicating removal of mannitol from the cement samples. The dissolution of mannitol was confirmed by XRD and FTIR analysis of samples before and after incubation. Prior to PBS incubation, XRD indicated the presence of MgHPO4, MgNH4PO4 and mannitol, as expected, with the mannitol no longer observed following PBS exposure. SSA measurements indicated decreased surface area with increased mannitol content after reacting and, as with the true density, the mannitol-containing cements normalized to similar to 0% mannitol following PBS incubation. Measurement of pH in PBS showed no significant difference between samples, with the pH remaining near 7.5 through 14 days. Of particular concern when introducing porosity to highly soluble cement is the potential for reduced cell viability due to increased surface area and dissolution. In **Figure 2** are preliminary cytocompatibility results which showed, qualitatively, no drastic drop in cytocompatibility with increased mannitol.

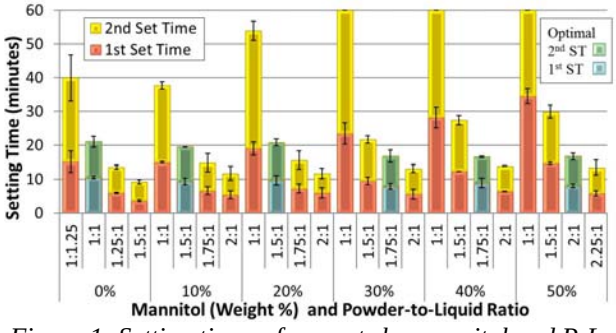

Figure 1: Setting times of cements by mannitol and P:L

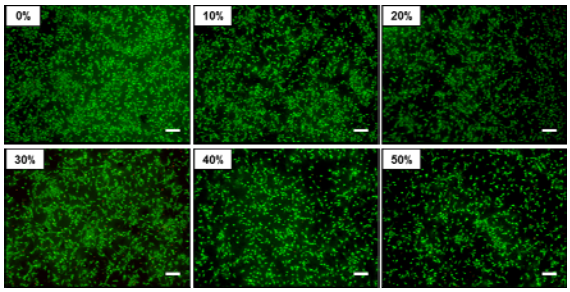

Figure 2: Live/Dead staining of MC3T3 cells on cements with 0-50% mannitol. [scale: 200um, 72 hours of culture]

Conclusions: Magnesium ammonium phosphate cements were successfully created with up to 50 weight % mannitol pore formers. Optimized powder-to-liquid ratios were chosen to yield clinically appropriate handling characteristics. All embodiments maintained a balanced pH and high cytocompatibility. Future studies will focus on mechanical strength, dissolution and cell infiltration.

References: [1] Hoffman MP. Acta Bio. 2009;5:43-49. [2]Mestres G. Acta Bio. 2011; 7:1853-1861. [3] Klammert U. J. Mater Sci: Mater Med. 2010; 21:2947-2953. [4] Wang AJ. Mater Sci Eng C. 2013;33:2508-2512. [5] Cama G. Acta Bio. 2009;5:2161-2168. [6] Xu HHK. Biomaterials. 2006;27:4279-4287.