Degradable Calcium Polyphosphate Compaction Strategies for the Delivery of Therapeutic Agents

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Introduction: Osteomyelitis is a difficult-to-treat bone infection usually caused by pyogenic bacteria. With an annual incidence of 2% in the United States, osteomyelitis results in substantial health care cost and disability [1]. A major challenge with the current standard treatment lies in maintaining sufficient bactericidal concentrations at the infected site while avoiding system toxicity of non-targeted organs [2], stimulating the development of an appropriate local delivery system. Previous work by our group has established a reproducible gelling protocol that allows for the incorporation of antibiotics within an amorphous calcium polyphosphate (CPP) matrix without disrupting its functionality [3]. Subsequent elution studies have shown that this gelling protocol effectively delays antibiotic release in an aqueous environment [4]. The objective of the current study was to incorporate an additional compaction step as part of the gelling protocol in order to optimize the release profile of the loaded antibiotic from these CPP matrices.

Materials and Methods: An amorphous CPP starting material was obtained as previously described [3]. Vancomycin (VM)-loaded CPP matrices were initially produced using a previously established gelling protocol (G1) involving exposure of a CPP/VM/ddH_2O paste contained within custom molds to ~100% relative humidity followed by drying [3]. The resulting G1 disks were ground in a planetary ball mill, and then sieved to achieve the desired particle size distribution. 135 mg of the resulting particulate was placed into a punch-die system and subsequently compacted using a Carver hydraulic press at 30, 113 or 452 MPa for a contact time of 5 minutes. The compacted disks then underwent a subsequent 3 or 5 hour re-gelling procedure (G2).

Elution assays were conducted out to three weeks (n=8 per group) in a Tris-buffered saline at 37°C under gentle agitation as previously described [5]. To assess VM activity, the minimum inhibitory concentration (MIC) of eluted VM was determined using a microdilution assay as per the National Committee for Clinical Laboratory Standards protocol as previously described [4].

Results and Discussion: The compaction re-gelling protocol developed in this study significantly affected the release of vancomycin from these CPP matrices, with higher cumulative VM release seen for G1 disks at all time points ($p<0.05$). G2 disks, unlike G1 disks, did not exhibit the intrinsic burst release of vancomycin during the initial 8 hours of the elution study (Figure 1). The elimination of the burst effect from the release profile of G2 disks can be associated with an increase in visual homogeneity and density, as well as more uniform VM distribution. Elution studies further confirmed that VM release above MIC from these G2 disks was feasible up to 456 hours (Figure 1), a clinically acceptable therapeutic range.

Throughout this study attempts at modulating release kinetics were made by altering compaction stress, particle size, and re-gelling time. No significant differences were noted, however, in release profiles for G2 disks compacted at either 113 or 452 MPa using particulate having a <45µm or 45-212 µm particle size distribution ($p>0.05$). Limited control of elution kinetics was demonstrated by attempting to create a disk that underwent a shorter controlled re-gelling time (3 hrs) with a decidedly larger particle size distribution (212 – 500µm) and a very low level of compaction (30 MPa). These disks physically resembled other G2 disks, but showed a slower release of vancomycin at early time points, with a significantly higher release during the latter stages of elution. These kinetic variations are best attributed to the particle size distribution, with the much larger G1 particulate likely delaying antibiotic release that may be linked to diffusional transport or matrix degradation.

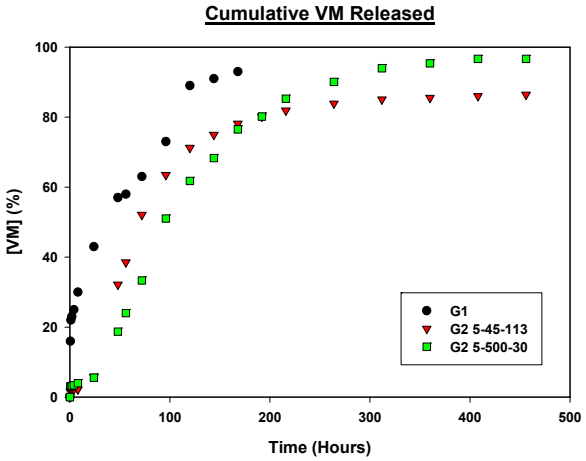

Figure 1: Cumulative release of vancomycin for a 456- hour elution study.

Microbiological activity assays confirmed that the stability of VM in the current elution studies was not significantly altered after 456 hours (MIC=2-4µg/mL). This minimal loss in potency (as-reconstituted MIC=1µg/mL) coincides with normal bactericidal loss of vancomycin in solution during that time frame, suggesting that its inherent bactericidal ability is effectively retained during matrix processing and subsequent elution.

Conclusion: Overall, the new compaction re-gelling protocol yielded more homogeneous CPP delivery matrices, significantly reduced the burst release phenomena, while extending therapeutic levels of vancomycin into a clinically acceptable range. Given the improved *in vitro* release kinetics and potential for process control as compared to many existing calcium phosphate delivery systems, *in vivo* characterization of this approach should be explored.

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