Strategies for Manipulating the Chain Length of an Amorphous CPP Delivery System

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Introduction

With the development of a low-temperature fabrication protocol involving stages of gelling and compaction, our research group has shown that Calcium Polyphosphate (CPP) is capable of loading and releasing thermal labile therapeutic agents with some limited success[1-3]. To provide even greater control of therapeutic release, the geometric versatility and structural integrity of the CPP delivery matrices needs further refinement. It was previously found that increasing the exposure time to humidity from 5hrs to 24hrs resulted in a small drop in chain length, and a subsequent increase in burst release and reduction in the duration of sustained antibiotic release *in vitro*[1, 3]. From such observations the question becomes whether we can further modulate this function by increasing chain length through various processing methods and, in so doing, improve the drug release from the resulting matrices. In this study two distinct fabrication methodologies that showed promise for favourably altering chain length were examined. The guiding hypotheses are that the chain length of CPP can be increased and that this increase will further extend the release of antibiotic from the delivery matrices.

Materials and Methods

Our standard amorphous CPP was produced following the protocol developed by Pilliar *et al*[4]. After washing the resulting amorphous CPP frit in 100% ethanol, the frit was then milled and sieved to obtain <45um fraction of particles. To achieve amorphous CPP of an assortment of chain lengths, we first fabricated CPP samples under extreme furnace conditions (50hr calcine, 1500°C melt, or 10hr melt). As a second fabrication route amorphous CPP was fabricated from sodium polyphosphate (NaPP). First, melt-derived NaPP was fabricated by heating sodium phosphate monobasic monohydrate in a Pt dish at 800°C for 20hrs. The melt was then rapidly quenched on a copper plate and cooled to room temperature. Aqueous solutions of this NaPP was next mixed with $CaCl_2$*$2H_2O$ to form a CPP precipitate. To optimize chain length via this second fabrication route NaPP concentration, presence of buffer (e.g. $N(CH_3)_4OH$) and order of reactant addition were manipulated. Vancomycin (VCM) – loaded CPP disks were fabricated following the protocol by Petrone *et al*[2]. Here, however, the first gelling phase lasted 2hrs, the compaction stress applied to the resulting G1 powder in the stainless steel moulds was 113MPa, and the second gelling segment was 3hrs in length. These final 'G2' disks were used in the *in vitro* elution study.

Composition of the as-made CPP powder was confirmed after dissolution in 6N HCl using ICP-OES (PerkinElmer Optima8000). Chain length was assessed using solid state ^{31}P NMR measurements on a spectrometer (Bruker Avance) and Dmfit 2010 software. For the *in vitro* elution study, 15mL of 0.1M TBS was added to each disk within a cornea viewing chamber on a horizontally rotating plate in a 37°C room[3]. At given time points 7mL of elution media was removed for measurement of the release of VCM, calcium, and phosphate. VCM release was measured using ultraviolet-visible spectrophotometer (BioTek Instruments Synergy HT) at a wavelength of 280nm. Phosphate and calcium release were analyzed by ICP-OES. The chain length and elution data was analyzed using Minitab15.0, a statistics software program, by one-way analysis of variance with a significance value of p=0.05. In addition, a post-hoc pairwise Tukey analysis was performed.

Results

We were unsuccessful in manipulating chain length using extreme furnace conditions, with results showing a drop in CPP chain length compared to the standard (control) melt protocol (where chain length is ~60). Meanwhile, the second process involving NaPP did not significantly alter chain length compared to the control CPP. However, concentration, reactant addition, and presence of a buffer did have an impact on the chain length of the precipitated CPP (Figure 1).

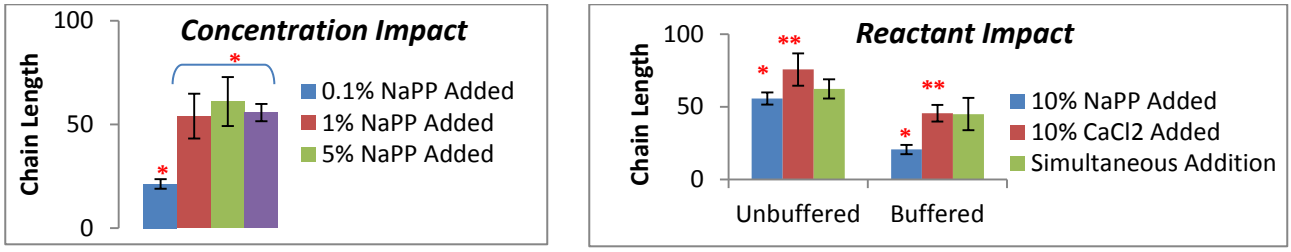

Figure 1: Impact of NaPP Concentration (left) and Order of Reactant Addition (right) on the Chain Length of the Precipitated CPP (N=3). Values reported as average ± one standard deviation (* denotes significant difference).

Adding 1, 5 or 10% NaPP had no significant effect; however, a 0.1% NaPP concentration resulted in a significant drop in CPP chain length. The addition of NaPP to $CaCl_2$ resulted in the lowest chain length of any of the reactant addition sequences, while buffering saw a further drop in chain length regardless of this sequence. ICP analysis confirmed that the Ca/P molar ratio for all CPP precipitates was consistently 0.40-0.50 and that the residual sodium was less than 6mol%.

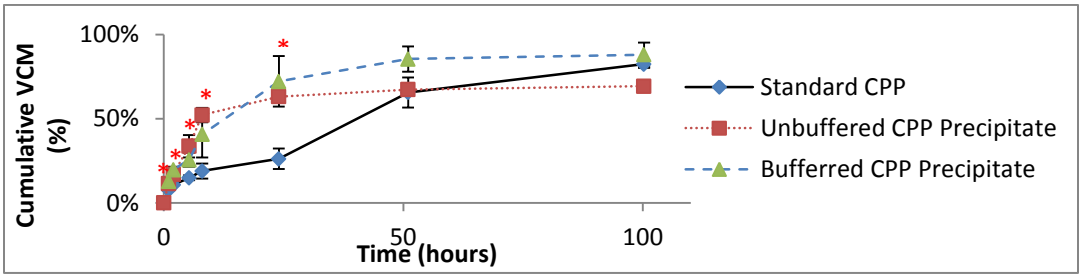

Figure 2: Cumulative Release Profiles of VCM from CPP G2 Disks (N=5). Values reported as average ± one standard deviation. (* denotes significant difference between standard CPP and Precipitates at time point).

As shown in Figure 2 the performance of CPP with similar chain lengths but different fabrication strategies exhibited significantly different VCM release profiles within the first 24hrs.

Discussion

Contrary to our initial hypotheses the chain length of CPP was not readily manipulated by either fabrication strategy. We did, however, show that even within the limited chain lengths achieved with precipitation that concentration, order of reactant addition, and presence of a buffer had an impact. It is possible that the higher chain length of NaPP did not translate to CPP as a result of the opposing actions of calcium sequestration by NaPP upon addition of $CaCl_2$ and hydrolytic degradation encouraged by displaced hydrogen. Lastly, fabrication methodology was found to influence the initial release behaviour of the VCM from the CPP matrices.

References

[1.] Dion et al.. *Biomater*. **2005**, 26, (21), 4486-4494. ; [2.] Petrone et al.. *Acta Biomater*. **2008**, 4, (2), 403-413. ; [3.] Dion et al. *Biomater*. **2005,** 16, (35), 7276-7285. ; [4.] Pilliar et al. *Biomater*. **2001**, 22, 963-972.