A Novel In-Situ Forming Calcium Polyphosphate System for Anti-Cancer Drug Release

Arash Momeni[1], Sean Curley[2], Mark Filiaggi[1,2]
[1]School of Biomedical Engineering and [2]Faculty of Dentistry, Dalhousie University, Halifax, Canada

Statement of Purpose: One major disadvantage of chemotherapy is the adverse side effects of the anti-cancer drugs because of widespread systemic exposure, making localized delivery very desirable method for treating these illnesses. We recently developed an injectable in-situ forming system with the potential to deliver and release drugs locally in procedures such as trans-arterial chemoembolization (TACE). In TACE, a biomaterial is administered directly into blood vessels feeding a tumor thereby blocking its nutrient supply and also releasing chemotherapeutics locally [1]. Our in-situ forming system is comprised of sodium polyphosphate and calcium chloride solutions which, on contact, form a coacervate or highly viscous liquid that is immiscible with water. The objective of this study is to demonstrate the capability of an optimized system to be loaded with anti-cancer drugs that can subsequently be released in a sustained manner.

Methods: Sodium polyphosphates with different degrees of polymerization (D_p) were prepared through condensation polymerization reaction of NaH_2PO4 and KH_2PO_4. A 1M $CaCl_2$ solution was used as the calcium source and doxorubicin, being routinely used for TACE, was chosen as the model drug. Doxorubicin was added to the sodium polyphosphate solution and then calcium chloride was mixed with this polyphosphate solution forming a coacervate instantly with some excess fluid on top. The first objective of this study was to optimize the system by minimizing the amount of free drug in the excess fluid. Using Design-Expert® software, a surface model analysis was developed with 25 design points to assess the effect and significance of polyphosphate D_p (short = 272, medium = 10,000, and long = 23,500), polyphosphate concentration (3-10 g/100mL), doxorubicin concentration (0.02-0.4 mg/mL) and overall calcium to phosphorus mole ratio (35-50%) on the ability of the gel to trap the highest percentage of the drug (%drug loading). Subsequently, long-chain and short-chain formulations with optimum drug loading capability were selected for use in the drug release study at 37°C. At each time point, 4mL of the 5mL of buffer (added following removal of the excess fluid) was removed for analysis and replaced with 4 mL of fresh buffer. Doxorubicin loading and release were determined by measuring solutions for absorbance at 480 nm using a UV/VIS spectrophotometer.

Results: Figure 1 depicts a representative calcium polyphosphate coacervate loaded with doxorubicin, which is red in color. A model with a good fit to design point data (insignificant lack of fit; p value=0.109) was generated, allowing navigation through the design space. All four variables significantly affected the %drug loading (p value < 0.003), with polyphosphate D_p and doxorubicin concentration being the most significant variables (p value < 0.0001). Figure 2 shows the quadratic model that was fitted to the experimental data representing the %drug loading against these two variables. Using this model a long-chain and a short-chain formulation were selected by the software to give the highest %drug loading (Table 1). Figure 3 describes the drug release profile of these two optimized samples.

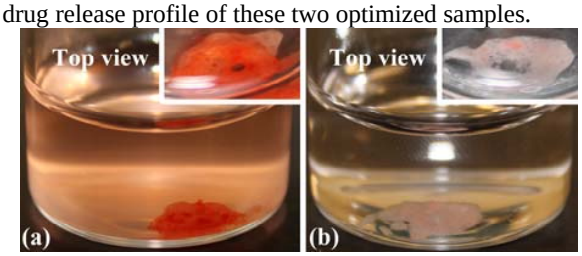

Figure 1: Images of a long-chain coacervate after (a) 26 and (b) 221 hrs

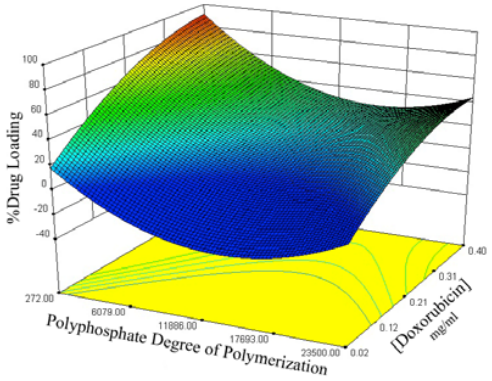

Figure 2: %Drug loading model produced by Design-Expert® for fixed [polyphosphate] (6.5 g/mL) and Ca/P mole ratio (42.5)

Table 1. Optimized samples and their drug loading results.

Polyphosphate D_p	Polyphosphate Concentration (g/100ml)	Doxorubicin (mg/ml)	Overall calcium to phosphorus mole ratio (%)	Predicted theoretical drug loading (%)	Actual experimental drug loading (%) (n=3)
Long chain-23500	3	0.34	42.17	53	53±2
Short chain-272	3	0.4	35	100	97±0.2

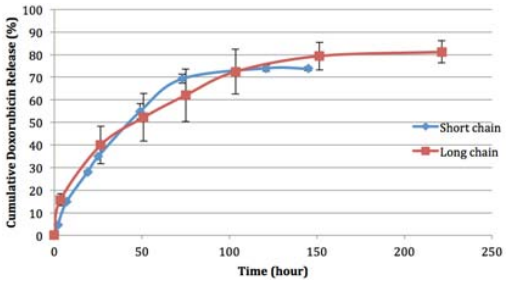

Figure 3: Cumulative release from optimized samples (n=3)

Conclusions: It was demonstrated that an in situ forming calcium polyphosphate system can be predictably and efficiently loaded with doxorubicin with a subsequent slow rate of release. Future work will include testing the cytotoxic properties of the released drug to confirm its stability and effect within the calcium polyphosphate coacervate.

References: 1. M Biolato, et al. *Eur Rev Med Pharmacol Sci* 2010; 14:356.