Fabrication of Chitosan-Alginate Microparticles Loaded with Simvastatin for Sustained Drug-release *in vitro*

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Statement of Purpose: Simvastatin (Sim) has been reported as a drug candidate in both chemotherapy [1] as well as bone regenerative therapy [2]. Depending on the patient's unique need and dosage, a targeted and sustained drug release system is essential. Microparticles (MPs) are an attractive option due to their potential to be bioinert, maintain physiological pH and function as a targeted drug release system. Sim has been shown to cause regenerative [2] effects on bone tissue but is a poorly water-soluble drug [3]; thus, a suitable solvent dissolution step is required to be integrated with the MPs fabrication. Moreover, the cationic nature of CS can be harnessed to attain a more stable release of Sim which has a comparatively lower isoelectric point than CS. In this study, Sim-loaded Chitosan-Alginate MPs were prepared with the aid of a simple polyelectrolyte complex-based method. The objective of this study is to be able to obtain a sustained drug release profile *in vitro* at physiological conditions.

Methods: 2% *w/v* CS (low molecular weight) solution was dissolved in 1% *v/v* Acetic Acid, stirred for an hour, and then filtered through a 52 µm nylon mesh. The CS solution was stirred along with various concentrations of Sim – 100 µg/ml, 500 µg/ml, 1000 µg/ml, and 5000 µg/ml. The Sim was dissolved in 200 proof ethanol before being transferred to the CS solution. A cross-linker solution of 1% *w/v* Sodium Tripolyphosphate and 1% *w/v* Sodium Alginate was prepared for coacervation. The CS-Sim solution was added dropwise via a 30 G needle into the cross-linker bath stirred at 300 rpm, and crosslinked MPs were removed after 30 minutes. The extracted MPs were rinsed and filtered once with DI Water using P5 filter paper. The obtained MPs were spread in a monolayer on a glass plate and allowed to dry under a fume hood overnight. For the drug release assessment, a UV Vis spectrophotometer (Jasco) was used to record the absorbance at 239 nm in a 1X Phosphate Buffered Saline (PBS) medium. Moreover, the pH variation due to the hydrolytic degradation of the MPs was assessed for maintaining adequate physiological pH (Mettler Toledo pH meter), especially for tissue regeneration engineering. The release study medium was made slightly enzymatic (lysozyme mixed at 0.001% *w/v*) to mimic *in vivo* conditions.

Results: The drug release was found to be sustained without a burst release during the entirety of the 24-day duration, as shown in figure 1, denoting that these Sim-loaded MPs are an excellent candidate for a targeted drug release system. Upon hydrolytic degradation starting at physiological pH and temperature, the pH was maintained near 7.4, as shown in figure 2. The MPs exhibited a relatively low swelling ratio with no significant difference with time at a 95% confidence interval.

Conclusion: This drug release system is promising for both chemotherapeutic and bone regenerative uses due to Simvastatin's drug of fit properties. Sustained release of Sim from the MPs can be attributed to the charge difference between the drug and the carrier, along with the poor water solubility of the drug itself. The release mechanism empowers these MPs to be used in therapeutic applications of Sim delivery in localized target areas, without hindrance to the physiological pH. Future studies may include coating the MPs in Gelatin to create a more nurturing environment for cell growth, with scope in the translation of these carriers in a clinical setting.

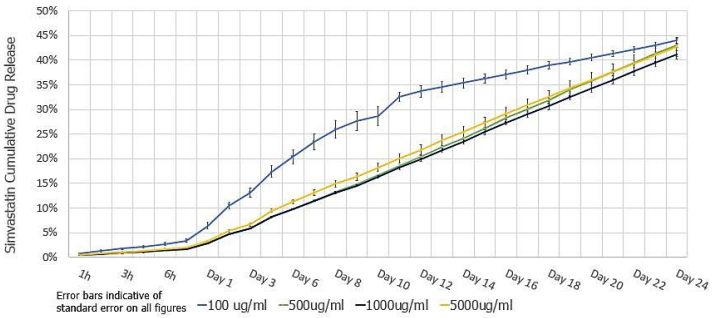

Figure 1 Sim release profile from the MPs. The release medium used was 1X PBS (2 ml), maintained at 37 ^{O}C and continuously shaken at 50 rpm. PBS was removed for analysis and freshly added to the vials for the next time point. Errors bars represent standard error from triplicates.

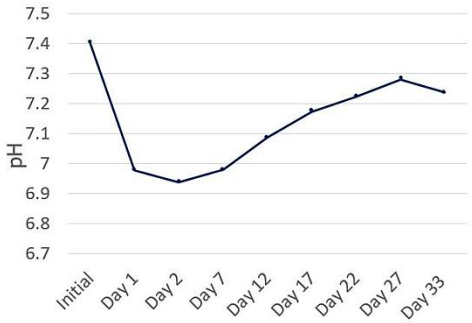

Figure 2 pH change during hydrolytic degradation of MPs. Lysozyme mixed 1X PBS was used as the study medium that was replaced at each time point.

References:

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[3] Zhang Y. et al. Int J Pharmac. 2011;410(1-2):118-124.