Silk-Elastinlike Polymers Enhance the Anti-inflammatory and Analgesic Properties of Semisynthetic Glycosaminoglycans

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Introduction

The bladder urothelium poses an obstacle for intravesical delivery of water-soluble drugs due to short residence time after installation and barriers to diffusion.[1] Thermoresponsive polymers can increase intravesical dwell time of water-soluble therapeutics and enhance their bioaccumulation profile in the bladder.[2] We evaluated four distinct classes of polymers for the influence of their physicochemical properties on their ability to enhance delivery of a semi-synthetic glycosaminoglycan ether (SAGE) GM-0111, a next generation anti-inflammatory anti-pain therapeutic, to the bladder via intravesical administration. Poloxamer 407, poly(lactic-*co*-glycolic) acid (PLGA)- poly(ethylene glycol) (PEG)-PLGA (1000:1000:1000 MW), silk-elastinlike protein polymers (SELP) 815K, and 415K were selected for evaluation based on each of their distinct properties.

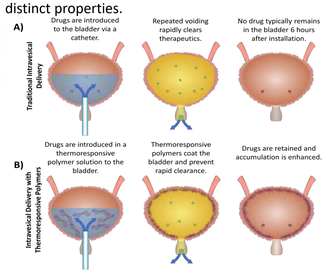


FIGURE 1: Schematic depiction contrasting the differences between traditional intravesical therapeutic administration to thermoresponsive polymer enhanced delivery. A) The current clinical standard for intravesical delivery is the administration of the therapeutic dissolved in saline. After, a specified period the catheter is removed and the therapeutic solution drained. Soon after, all of the therapeutic moiety is cleared from the bladder halting any further accumulation or maintenance of therapeutic effect. **B)** After installation of the thermoresponsive polymer solution it will undergo a phase transition precipitate out of solution forming an insoluble matrix within the bladder. This entraps the therapeutic and enhances its retention within the bladder promoting increased efficacy.

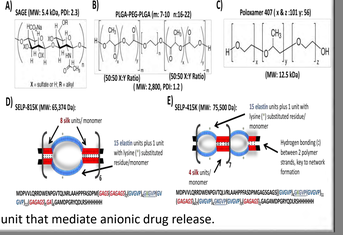


FIGURE 2: Structures of polymeric materials used in this study. A) The therapeutic SAGE. **B)** PLGA-PEG-PLGA **C)** Poloxamer 407 **D) & E)** Graphical depictions of SELP 815K- and SELP-415K structures, respectively. The single letter amino acid code for each polymer is included below the structures. Red represents **silk-like** units forming a rigid backbone, and blue signifies the flexible **elastin-like** units that allow for poreformation, : is used to convey the hydrogen bonding between the silk-like units which gives the gels their mechanical strength, and the green asterisk indicates the positively charged lysine substituted elastin-like unit that mediate anionic drug release.

In Vitro Characterization of Materials

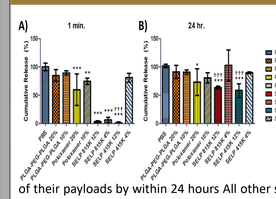
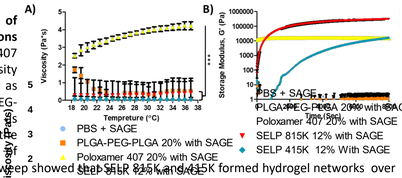


FIGURE 4: Comparison of burst release and total release of SAGE from thermoresponsive polymers. A) Burst release from the polymeric carriers at high and low concentrations. *, **, and *** indicate p-values of less than 0.05, 0.01, and 0.001 when sample is compared to the PBS control. ††† indicates a p-value of less than 0.001 for comparison of the high and low concentrations polymer. **B)** Cumulative release after 24 ht. SELP 815K and 415 released 63% ±3% and 58% ±12% of their payloads by within 24 hours All other samples released between 80-100% of their payload within 24 hours.

FIGURE 5: Rheological evaluation of thermoresponsive polymer solutions loaded with SAGE. A) Poloxamer 407 significantly increased its viscosity compared to other polymers as temperature increased. PLGA-PEG-PLGA decreased its viscosity as temperature rose, likely due to the polymer precipitating out of solution **B)** The oscillatory time sweep showed that SELP 815K and 415K formed hydrogel networks over time. Poloxamer 407 also demonstrated a high storage modulus while PLGA-PEG-PLGA was statistically indistinguishable from their payloads by within 24 hours the PBS control. The SELP systems increased in modulus over time. However, the SELP 415K was in a solution state for longer than the SELP 815K.



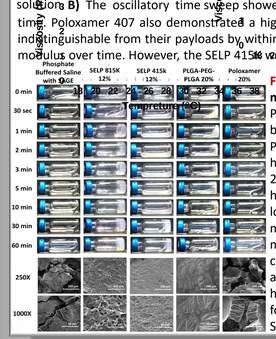


FIGURE 6: Visual assessment of gelation kinetics at 37 °C and microscopic structure of gels. SAGE at 10 mg/ml does not gel. PLGA-PEG-PLGA demonstrated a rapid increase in turbidity, but then phase separated into a solid film and a liquid phase. Poloxamer 407 did not form hydrogels, but transitioned into a highly viscous fluid. SELP 815K 12% formed a hydrogel within 2 minutes at 37 °C. SELP 415K 12% took 30 min. to gel and had an observable phase separation. The scale bars in the lower right of each photograph represent 5 mm. The materials were lyophilized and imaged using electron microscopy. PLGA-PEG-PLGA was a highly viscous polymer coating with a thick skin after lyophilization. Both SELP 815K and 415K formed robust porous networks, with the 415K having substantially smaller pores. This more dense structure for 415K likely contributed to the slower observed release of SAGE from the matrix.

Effects on Pain and Inflammation

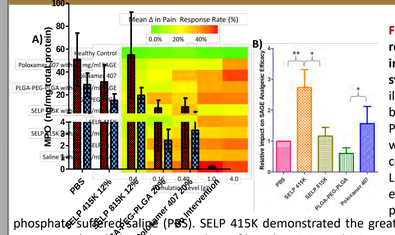


FIGURE 7: Assessment of pain response in a LL-37 induced model of interstitial cystitis and Painful bladder syndrome (IC/PBS). A) A heat map illustrating the changes pain response between SELP 815K, SELP 415K, PLGA-PEG-PLGA, Poloxamer 407, and saline with and without SAGE. A healthy control arm that never was exposed to LL-37 was included. **B)** The relative enhancement of analgesic effect the polymer generated compared SAGE to phosphate buffered saline (PBS). SELP 415K demonstrated the greatest enhancement of SAGE's analgesic properties. * and **, indicate p-values of less than 0.05 and 0.01, respectively.

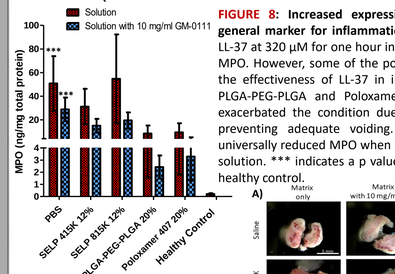


FIGURE 8: Increased expression of Myeloperoxidase (MPO), a general marker for inflammation, is decreased by SAGE GM-0111. LL-37 at 320 µM for one hour induced a highly significant elevation of MPO. However, some of the polymers seemed to interact to reduce the effectiveness of LL-37 in inducing inflammation, most notably PLGA-PEG-PLGA and Poloxamer 407. SELP 815K seems to have exacerbated the condition due to impairing bladder function and preventing adequate voiding. However, the inclusion of SAGE universally reduced MPO when administered regardless of the carrier solution. *** indicates a p value of less than 0.001 compared to the healthy control.

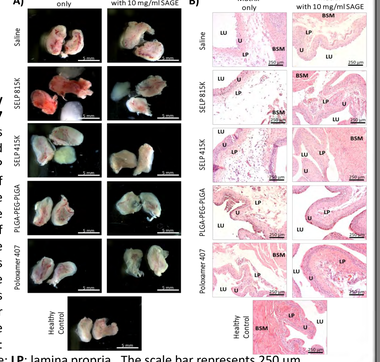
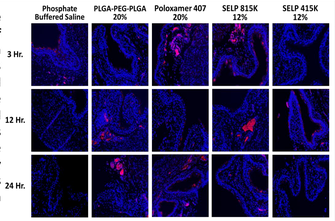


FIGURE 9: Gross anatomy and Histology of the bladder 24 hours post LL-37 insult. A) The hemisected bladders showed erythema in all bladders that did not receive SAGE. In the saline and SELP 815K groups there were signs of hemorrhage and tissue edema. The addition of SAGE drastically reduced the erythema and other visual signs of inflammation almost to the level of the healthy control. The scale bar represents 5 mm. **B)** Histology supported the observations made in the gross anatomical observation. BSM, bladder smooth muscle layer. The labels used are as follows **LU**: lumen of the bladder; **U**: urothelium; **BSM**: bladder smooth muscle; **LP**: lamina propria. The scale bar represents 250 µm.

Effects on Bladder Function

FIGURE 10: Representative bladder tissue samples showing enhanced accumulation of SAGE. SAGE accumulation is enhanced when delivered via thermoresponsive polymers at 3 hr, 12 hr and 24 hr after installation compared to PBS controls (left column) . All of the thermoresponsive polymers showed increased retention after 12 and 24 hours which confirms our hypothesis that the SAGE would become entrapped and retained within the bladder by the polymers. The **SAGE GM-0111-CF™633** is shown in red. The bladder tissue stained with **Hoechst 3342** is shown in blue.



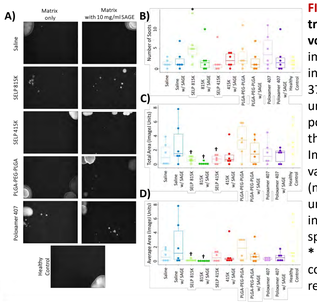


FIGURE 11: Assessment of bladder function after treatment with thermoresponsive polymers via a voiding spot assay. A) Representative fluorescent images of the filter paper placed under an individual mouse's cage 24 after treatment and LL-37 induced bladder insult. Clear alterations in urinary behavior are observed based upon the polymer used. **B)** A box and whisker plot depicting the number of unique void spots detected in the ImageJ counting algorithm. Each point marks the value of an individual mouse within each group (n=6). **C)** The total area of the filter covered with urine, which corresponds the total urine excreted in the 2 hour period. **D)** The average area of the spots, which is a measure of average void volume. * and † indicate p values less than 0.05 when compared to the saline and healthy control groups, respectively.

Discussion and Conclusions

These results show that thermoresponsive polymers enhance intravesical delivery of water soluble therapeutics to the bladder. Matrix formation was not critical to enhance accumulation but did improve residence time. Care needs to be taken to insure the polymers themselves, such as was the case with SELP 815K, do not exacerbate disease conditions. SELP 415K demonstrated the greatest potential for enhancing SAGE GM-0111's therapeutic effects while not causing any significant impairment to bladder function. 415K's success over 815K can be attributed to slower gelation and greater elasticity. Improved understanding of polymer properties and their potential to enhance intravesicular deliver of drugs may improve intravesical treatments for bladder cancer, interstitial cystitis, and painful bladder disease.

Methods

Material Preparation

- SELP 815K and 415K were synthesized and purified as described previously [3], and the synthetic polymers were obtained from Sigma Aldrich. SAGE GM-0111 was obtained from Glycomira (Salt Lake City, Utah). PLGA-PEG-PLGA and Poloxamer 407 were prepared at 20% and 10% by weight by sonicating in an ice bath for two hours. SELP 815K and SELP 415K 12 wt/wt% solutions were thawed from previously prepared stocks and 4 wt/wt% solutions were obtained by diluting the SELP with phosphate buffered saline (PBS). SAGE GM-0111 was added at 10 mg/ml to each solution.

In Vitro Evaluation

- Release from PLGA-PEG-PLGA, Poloxamer 407, SELP 815K and 415K was measured in Surine® simulated urine without enzyme. Polymer concentrations were reported as the weight percent. Each polymer solution was loaded with 10 mg/ml SAGE GM-0111. Cumulative release was quantified via a colorimetric azure A assay by measuring the change in absorbance at 620 nm from background (n=6).
- Polymer solutions with 10 mg/ml SAGE were loaded into chilled hermetically sealed glass vials and kept on ice. Vials were then tilted 90° and imaged using a digital camera (T=0). They were then placed in a 37 °C water bath and imaged again after 30 sec, 1 min, 2 min, 3 min, 5 min, 10 min, 30 min, and 60 min. Based on observed gelation and SAGE release profiles, PLGA-PEG-PLGA 20%, poloxamer 407 20%, SELP 815K 12%, SELP 415K 12% were selected for rheological evaluation. A viscosity sweep from 18 to 37°C was obtained under 0.1% strain and a 3 hr oscillatory time sweep (6.283 rad/s) was performed at 37°C to monitor the storage modulus (G'), loss modulus (G"), and gelation kinetics.
- Samples were allowed to cure at 37 °C overnight, flash frozen in liquid nitrogen, and lyophilized. Prior to imaging them on a FIE Quanta 600 SEM, a 10 nm gold palladium sputter coat was used to reduce sample charging.

In Vivo Assessment of Intravesical Delivery

- We used 8week old female C57Bl/6 mice and administered each treatment as shown in Figure 3.
- Fluorescently labeled SAGE GM-0111-CF™633 was used to asses distribution in healthy mice sacrificed at 3 hr, 12 hr, and 24 hr after intravesical administration (n=3 per group per time point) and the bladders imaged at 40x magnification on an Olympus FV1000 confocal laser scanning microscope.
- Mice were given 50 uL of test polymer with or without 10 mg/ml of SAGE GM-0111 intravesical. Then an Interstitial cystitis/ painful bladder syndrome (IC/PBS) like-state was induced via the intravesicular administration of 320 µM LL-37 through the catheter for 1 hr. The gross anatomy, histology, suprapubic pain response, MPO concentration and bladder function were assessed as previously described.[4-5]

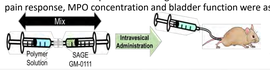


FIGURE 3: Graphical depiction for incorporation of thermoresponsive polymers for intravesicular delivery.

Statistics

- Graphs and charts were created in GraphPad Prism™ 5.0 or R-Studio. Data is reported as the mean ± standard deviation unless otherwise specified. A one-way analysis of variance with Bonferroni post-test was used to compare significance between multiple groups.

Acknowledgements

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