Modulation of Polymer/DNA Release from Poly(lactic-co-glycolic acid) Microspheres through Poly(ethylenimine) Modification and Loading Concentration

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Statement of Purpose: One of the major characteristics of polymeric gene delivery is the transient nature of transfection. In many cases, this limits the desired therapeutic effect of the delivered nucleotides. One potential method to extend the duration of these transient responses is through the controlled release of polymer/DNA (either bPEI/DNA or bPEI-HA/DNA) complexes from microparticles. The objective of this study was to determine the effects of microparticle inner phase concentration and branched poly(ethylenimine) (bPEI) modification with hyaluronic acid (HA) on the encapsulation efficiency, release rate, and *in vitro* transfection efficiency in a poly(lactic-co-glycolic acid) (PLGA) microparticle system. We hypothesized that HA modification would significantly alter the release profile while increasing transfection efficiency.

Methods: Commercially available HA, branched bPEI, and 50:50 PLGA were used in the work described below. HA was conjugated to bPEI through a reductive amination reaction. bPEI and bPEI-HA were then tagged with rhodamine to allow for effective tracking. Then, either plasmid DNA encoding for eGFP alone, bPEI/DNA complexes, or bPEI-HA/DNA complexes were created and loaded into PLGA microparticles formed using a water-in-oil-in-water emulsion. A set internal phase volume was used with three varying concentrations (0.25 (low), 0.75 (med), and 1.25 (high) mg/ml) of DNA and a constant N:P ratio of 7.5:1 for groups containing polymers. Once dry, the microparticles were washed, lyophilized and used for analysis. Release of both the polymer and DNA from each microparticle formulation was tracked over a period of 28 days via a previously established protocol[1]. Release was tested at 6 and 12 hours, and 1, 2, 3, 4, 7, 10, 14, 17, 21, and 28 days. Additionally, entrapment efficiency was examined via an extraction process and particle morphology was observed with SEM. Finally, transfection efficiency for each of the polymer containing groups was examined in 1 week intervals over a 28 day study period. For this, supernatant from 50mg/ml of microparticles in PBS from each test group was collected at days 7, 14, 21, and 28 and added to CRL1764 rat fibroblasts. The fibroblasts were then allowed to incubate for 72 hours before the cells were collected, fixed, and tested in a flow cytometer for transfection.

Results: Correct conjugation of the HA to bPEI was confirmed through NMR. Microparticle morphology and size did not vary significantly between any of the test groups. DNA encapsulation efficiency varied from a maximum of 65.5±7.1% in the case of the Low DNA concentration only group to 2.5±4.40% in the case of the High bPEI complex concentration group. As the inner phase concentration increased, the DNA encapsulation decreased. Polymer encapsulation varied from 63.7±2.1% in the case of the low bPEI-HA complex concentration group to 2.7±1.0% in the case of the high bPEI complex group. The polymer encapsulation also followed the trend discovered in the DNA encapsulation in that as concentration increased, the encapsulation efficiency decreased.

Release was monitored over 28 days and significant trends were identified within the groups. The modification of bPEI with HA accelerated the sustained release phase of DNA significantly. The cumulative DNA release for each group is shown below in figure 1. Interestingly, polymer release was not coupled to DNA release and showed a more significant burst release when modified with HA. The loading concentration had limited effects on the release profiles.

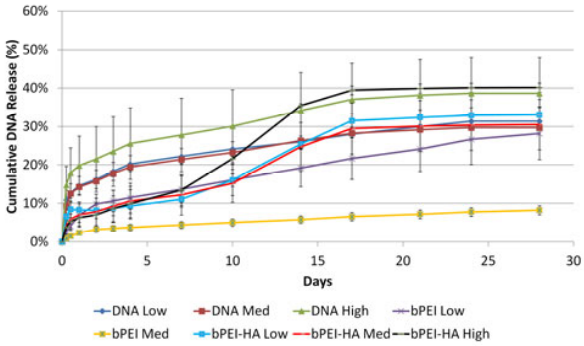

Figure 1. DNA Release Kinetics over 28 Days

Transfection efficiency validated the effectiveness of the released polymer and DNA. Significant transfection was observed with the first two weeks of release supernatant and decreased over the final 2 weeks. This agrees with the data observed in the release profiles as most of the DNA is released by day 17. Significantly, the highest observed transfection was seen in the second week of the high bPEI-HA complex loading group.

Conclusions: These results show that the modification of bPEI with HA and the concentration of loaded polymer/DNA complexes can significantly alter the entrapment and release profiles from PLGA microparticles. The HA in this system most likely accelerated the degradation of the particles resulting in this increased release[2]. This is significant in that it offers insight into the effects of modification of gene delivery vectors on a controlled release system designed to achieve a sustained therapeutic response. Additionally, the zwitterionic nature of the bPEI-HA polymer resulted in increased complex encapsulation in this system. The use of HA drastically altered and arguable improved the overall effectiveness of this system. Understanding these results can result in enhanced delivery of therapeutic DNA from engineered microparticles.

References:

1. Mountziaris P. Pharm Res. 2011;28(6) 1370-1384.
2. Lee E S. Colloid Surface B. 2007;5 125-130.