Intravaginal Gel for the Targeted Delivery of siRNA to T-cells as a Potential Strategy for HIV-1 Prevention

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ABSTRACT SUMMARY
The goal of this study is to develop and characterize a T-cell targeted nanomedicine for the active delivery of small interfering RNA (siRNA), which targets the viral genes or host factors involved in HIV-1 infection. This drug delivery system is designed for intravaginal administration as a potential pre-exposure prophylaxis to help women defend against HIV-1.

siRNA can be efficiently encapsulated into nanoparticles (NPs) with desirable particle size and release profile and siRNA-loaded NPs can be further conjugated with antibody for active T cell targeting. Resulting NPs are formulated into a vaginal gel dosage form to provide ease in self-administration and enhance retention within the vaginal tract.

INTRODUCTION
Human immunodeficiency virus/ acquired immunodeficiency syndrome (HIV/AIDS) is considered to be one of the most significant global health concerns in the 21st century [1]. Current HIV incidence appears to have a disproportionate impact on women [2]. Latest data revealed that women account for more than half of the infected population with unprotected sexual intercourse as the major mode of HIV transmission [2]. During the early stage of establishing vaginal entry and infection, HIV substantially invades intraepithelial vaginal Langerhans cells (a type of dendritic cells) and CD4+T cells through trauma or epithelial transcytosis [3]. Therefore, delivering preventative agents such as siRNA to target host or viral factors in these HIV-targeted cells may be an effective strategy to control and prevent HIV [4]. Carriers have been developed for the targeted delivery of siRNA into dendritic cells [5] and T cells [6]; however, none have focused on intravaginal targeted delivery. Therefore, our objective is to develop an intravaginal T-cell targeted system to deliver siRNA as a potential strategy for HIV-1 prevention.

EXPERIMENTAL METHODS
Non-specific siRNA was used as a model drug for the study of our drug delivery system. siRNA was first condensed by polyethyleneimine (PEI) and then encapsulated into NPs by a double-emulsion evaporation method using the biodegradable di-block copolymer, poly(lactic-co-glycolic acid)- polyethylene glycol (PLGA-PEG). Particle size and zeta potential were characterized by dynamic light scattering. Encapsulation efficiency (EE%) was determined with fluorescence-labeled siRNA using fluorescence spectroscopy. Release studies were determined in PBS (pH 7.4) at 37 °C. NPs were conjugated to anti-human anti-CD4 antibody (Ab) via the activation of N-Hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. Antibody conjugation efficiency (ACE%) was determined by subtracting unconjugated antibody from the total. Resulting antibody-conjugated NPs (NP-Ab) were then formulated into a 1% hydroxyethyl cellulose (HEC) vaginal gel and the NP-loaded gel was characterized in terms of viscosity and NP release from the gel. Student’s t-test (unpaired, two-sample, unequal variance with two-tailed distribution) was performed on all results with P <0.05 considered significant. Data shown are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION
The formulation of siRNA-loaded NPs was optimized in terms of particle size, zeta potential, EE% and release profile. The results showed that NPs formulated with 20 mg/mL of polymer had the smallest particle size and highest EE%; however, this formulation failed to achieve a sustained release profile (data not shown). NPs formulated with 10 mg/mL and 5 mg/mL of polymer had slightly larger particle size and decreased EE% compared to 20 mg/mL (Table 1), but both achieved sustained release of siRNA up to 13 days (Fig. 1). Release of siRNA from the 5 mg/mL formulation was higher than that of the 10 mg/mL formulation due to the decreased amount of polymer encapsulated.

Table 1. Particle size, zeta potential and encapsulation efficiency (EE%) of siRNA-loaded NPs, (N=3).

<table>
<thead>
<tr>
<th>C&lt;sub&gt;PLGA-PEG&lt;/sub&gt; (mg/mL)</th>
<th>Size (nm)</th>
<th>Zeta Potential (mV)</th>
<th>EE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>189.7±10.2</td>
<td>44.92±3.30</td>
<td>81.7±4.3</td>
</tr>
<tr>
<td>10</td>
<td>200.9±8.3</td>
<td>24.95±5.55</td>
<td>63.0±5.7</td>
</tr>
<tr>
<td>5</td>
<td>278.2±32.6</td>
<td>13.62±4.37</td>
<td>55.5±9.6</td>
</tr>
</tbody>
</table>

Table 2. Particle size, zeta potential and antibody conjugation efficiency (ACE%) of siRNA-loaded NPs, (N=3).

<table>
<thead>
<tr>
<th>ng Ab/µg NP</th>
<th>Size (nm)</th>
<th>Zeta Potential (mV)</th>
<th>ACE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>189.7±10.2</td>
<td>44.92±3.30</td>
<td>-</td>
</tr>
<tr>
<td>0.08</td>
<td>227.9±6.6</td>
<td>33.30±1.15</td>
<td>23.8±3.5</td>
</tr>
<tr>
<td>0.8</td>
<td>231.5±7.8</td>
<td>35.09±2.22</td>
<td>37.7±4.2</td>
</tr>
<tr>
<td>8</td>
<td>225.0±4.9</td>
<td>31.78±2.35</td>
<td>76.4±2.7</td>
</tr>
</tbody>
</table>

There was a positive correlation between the amount of antibody added and the amount of antibody conjugated to the NPs (Table 2). Antibody-conjugated NP (NP-Ab) achieved higher intracellular uptake of siRNA in the T-cell line Sup-T1 compared to unconjugated NPs (Fig. 2).
Vaginal gel consisting of 1% HEC and loaded with NP-Ab showed a non-Newtonian shear-thinning behavior and the viscosity of the NP-Ab loaded gel was comparable to over-the-counter lubricant gel products (Fig. 3). Approximately 20% of loaded NPs were released from the gel over 24 h (Fig. 4).

**Figure 1.** In vitro release profile of siRNA from NPs formulated with PLGA-PEG (N/P ratio=6/1) at 37 °C in PBS (pH 7.4). (A) siRNA-PEI PLGA-PEG NP formulated with C_{PLGA-PEG} of 10 mg/mL; (B) siRNA-PEI PLGA-PEG NP formulated with C_{PLGA-PEG} of 5 mg/mL; Values represent the mean±S.D., N=4.

**Figure 2.** In vitro cell uptake study of siRNA-PEI PLGA-PEG NP-Ab in Sup-T1 cells. Images were taken 24 hr post-treatment. Green: Cy3-labeled siRNA, Blue: DAPI

**Figure 3.** Steady-state flow curves of 1% HEC placebo gel and 1% HEC gel loading siRNA-PEI NP-Ab (1 mg NPs/g gel) with a single measurement at 37 °C.

**Figure 4.** In vitro release profile of NPs (formulated with 10 mg/mL PLGA-PEG) from 1% HEC gel at 37 °C in PBS (pH 5.0). Values represent the mean±S.D., N=4.

**CONCLUSION**

Our research group has developed a novel intravaginal nano-based drug delivery system for the active delivery of siRNA to T cells. siRNA can be efficiently encapsulated into PLGA-PEG NPs with desirable particle size for intravaginal delivery and sustained drug release. NP-Ab can be efficiently taken up by T cells. NP-Ab can be formulated into a gel dosage form that is comparable to marketed vaginal gel products.

**ACKNOWLEDGMENTS**

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**REFERENCES**