Biodegradable Intravaginal Film for the Targeted Delivery of siRNA-Loaded Nanoparticles to Immune Cells

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ABSTRACT SUMMARY
A novel intravaginal film platform was developed and characterized for the delivery of short interfering RNA (siRNA)-loaded nanoparticles (NPs) as a potential strategy for the prevention of sexually transmitted HIV infections.

INTRODUCTION
Vaginal drug delivery for the treatment and prevention of various viral infections has received increasing attention from pharmaceutical scientists and clinicians. Particularly, the use of RNA interference (RNAi) for knocking down gene expression has demonstrated great potential. Unfortunately, intravaginal delivery of siRNA has several challenges such as: inefficient transfection efficiency, non-specific targeting, and rapid removal from the vaginal tract. Nanoparticles can help overcome some of these obstacles and improve therapeutic efficacy. Vaginal films have several advantages over conventional vaginal dosage forms such as gels or tablets, which include portability, convenience in application, prolonged retention time, homogenous drug distribution, and easy storage. Intravaginal mucosal delivery of siRNA from biodegradable and biocompatible films is a promising approach to improve localized transfection efficiency within the local microenvironment.

Synaptosome-associated 23-kDa protein (SNAP23) is a soluble N-ethylmaleimide–sensitive factor attachment protein receptor (SNAREs) protein located on the plasma membrane and is important for human immunodeficiency virus type 1 (HIV-1) particle production. Hence, using specific siRNA against SNAP23 may disrupt the host SNARE machinery resulting in a significant reduction in HIV-1 virus particle production. For our system, the topical administration of film loaded with siRNA-encapsulated NPs against a pathologically relevant host cell target can knockdown the SNAP23 gene in immune cells and reduce the probability of HIV infection.

EXPERIMENTAL METHODS
PEG-PLGA/PEI/siRNA nanoparticles (NPs) were fabricated by a solvent evaporation method and functionalized with anti-HLA-DR antibody (Ab-NPs) for targeting HLA-DR+ dendritic cells (DCs). The optimized polymeric film embedded with siRNA loaded NPs was prepared by a solvent casting and evaporation method consisting of poly vinyl alcohol (PVA) and λ-carrageenan.

To examine the cellular uptake of siRNA loaded NPs, activated KG-1 immune cells were treated with NP/FAM-siRNA or anti-HLA-DR-NP/FAM-siRNA formulations. The cellular uptake of NPs was confirmed by microplate reader and fluorescence microscopy.

To examine the silencing activity of SNAP23 siRNA in vitro, KG-1 cells in complete medium at a density of 3×10⁷ cells/well were treated with SNAP23 siRNA NPs, antibody conjugated SNAP23 siRNA NPs, siRNA-NPs-in-films or antibody conjugated SNAP23 siRNA NPs-in-films at 37°C for 3 hrs. Lipofectamine2000 was used as positive control. RT-PCR was performed to determine gene expression and GAPDH was used as a housekeeping gene.

RESULTS AND DISCUSSION
The surface morphology of NPs and anti-HLA-DR conjugated NPs were examined by scanning electron microscopy (SEM) and representative images are shown in Figure 1.
Targeting activity of NPs were evaluated in differentiated KG-1 cells (elevated expression of HLA-DR) (Figure 2). Anti-HLA-DR conjugated NPs showed higher targeting activity than the NPs without conjugation. The intracellular levels of FAM-siRNA in KG-1 cells treated with anti-HLA-DR conjugated NP were highest after 3 h.

Finally, the therapeutic potential of our system was determined by measuring SNAP23 knockdown (Figure 3). Our results show that our Anti-HLA-DR conjugated NP-loaded film was able to knockdown the expression of SNAP23 by 31% in comparison to controls.

![Figure 1](image1.png)

**Figure 1.** Surface morphology of various film formulations. (a) blank film (b) siRNA/PEG-PLGA NP-film (c) SEM image of a blank film (d) SEM image of a siRNA-loaded film (e) SEM image of a siRNA-PLGA NP-film (f) SEM image of a siRNA-PLGA-PEG NP-film. SEM images were taken at ×100 magnification; inset images were taken at ×1000 magnification.

![Figure 2](image2.png)

**Figure 2.** Cellular uptake efficiency of the NPs and anti-HLA-DR-NP on mature KG-1 cells. (A) Representative fluorescence images (B) Quantitative analysis the cellular uptake of the fluorescent FAM-siRNA loaded NPs.

![Figure 3](image3.png)

**Figure 3.** RT-PCR analysis of SNAP23 gene expression. SNAP23 expression normalized to GAPDH. (*: P<0.05, n=3)

**CONCLUSION**

The vaginal film containing anti-HLA-DR conjugated PLGA-PEG/PEI/siRNA NPs were successfully developed and assessed in vitro as a carrier for siRNA. The films were homogeneous and transparent, with desirable physical properties. This novel NP-film formulation demonstrates potential utility as a microbicide platform to enhance vaginal siRNA delivery and for the prevention of HIV infection; however, further in vivo studies are required.

**REFERENCES**


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