Segmented intravaginal ring co-delivering hydroxychloroquine and siRNA-encapsulated nanoparticles for preventing HIV infection

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Introduction

Microbicides are an excellent alternative to condoms to help reduce transmission of human immunodeficiency virus (HIV). An intravaginal ring (IVR) would be a suitable platform that can provide controlled delivery of drugs within the female genital tract with high patient acceptance. We propose to develop a segmented combination IVR whereby one-half of the IVR will be loaded with hydroxychloroquine (HCQ), an immuno-modulatory drug that can induce a quiescent state in T cells and the other half will be coated with a pH-responsive film for the rapid release of small interfering RNA from nanoparticles formulation (siRNA-NP) triggered by an increase in vaginal pH due to the presence of seminal fluid as a novel strategy for protecting against HIV infection. The siRNA will knockdown the CCR5 gene expression, a co-receptor involved in HIV-1 infection.

Objectives

Development and characterization of pH-responsive film coated matrix IVR containing siRNA nanoparticles and reservoir-type IVR loaded with HCQ

Methods

- Solid lipid nanoparticles composed of glyceryl monosterate and L-α-phosphatidylcholine will be used to encapsulate siRNA using a double emulsion method to form siRNA-NP.
- siRNA-NP will be mixed with a pH-sensitive polymer (Eudragit L100) and used to coat a matrix-type polyurethane IVR segment, fabricated by hot-melt injection molding.
- HCQ will be loaded in a reservoir-type polyurethane IVR segment.
- Release studies will be performed in vaginal fluid simulant at basic and acidic pH.
- Cytotoxicity of the IVR segments will be evaluated using cervicovaginal epithelial cells.
- The CCR5 gene expression level will be evaluated by real-time PCR.

Results

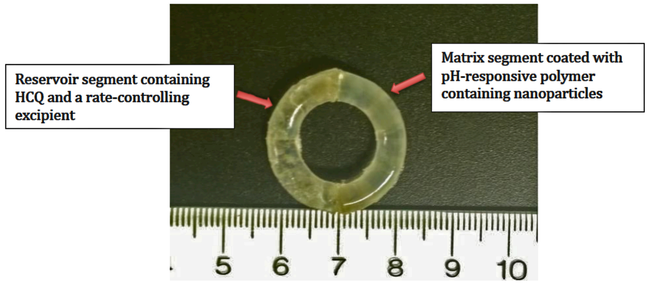

Fig 1: Segmented intravaginal ring (IVR). Picture depicts an IVR that is suitable for implantation in non-human primates (25 mm x 5 mm).

Size (nm)	Encapsulation Efficiency (%)	Zeta potential (mV)
219±35	85.95	-20±4.5

Table1: SLN Nanoparticles size, zeta potential and encapsulation efficiency

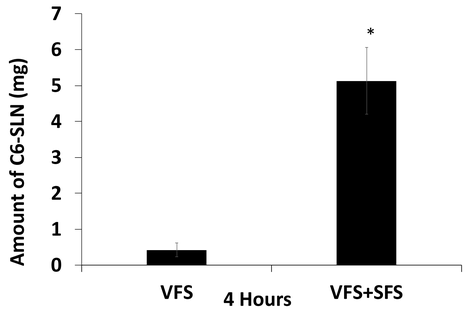

Fig 2: Release study of C6-SLN in VFS (vaginal fluid simulant) only and in VFS plus SFS (seminal fluid simulant) to simulate the presence of seminal fluid during sexual intercourse. Data represents the mean ± SD (n = 4). *P < 0.05 versus VFS. C6 coumarin-6.

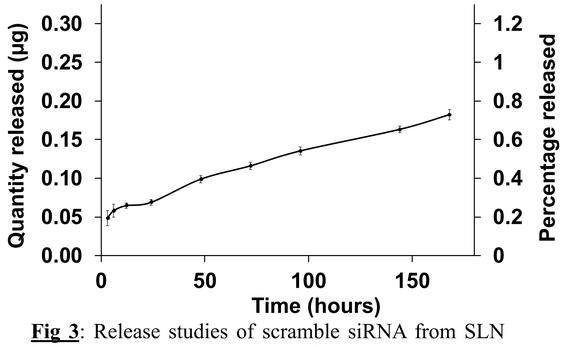

Fig 3: Release studies of scramble siRNA from SLN in PBS, Data represents mean +/- S.D. ; N=3

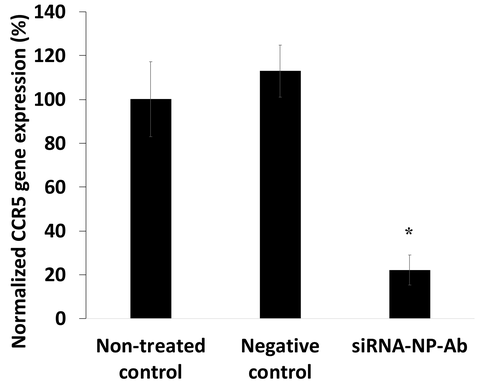

Fig 4: CCR5 gene knockdown in Sup-T1 cells incubated with IVR segments coated with pH-responsive Eudragit L100/PEG containing siRNA-NP-Ab (~ 19.09 µg of anti-CCR5 siRNA). Blank: Sup-T1 cells alone; negative control: consists of the IVR segment coated with the pH-responsive polymer but with drug-free SLNs. Data represents mean ± S.D.; N = 3. *P < 0.05 versus negative control

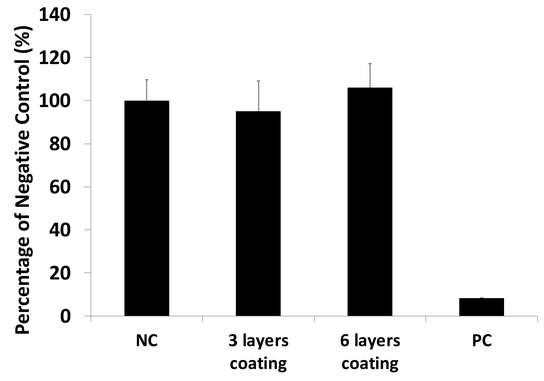

Fig 5: Impact of IVR coated with L100 on vaginal cells VK2/E6E7. Positive control (PC) = 1 M acrylamide and Negative control (NC) = medium. Data represent mean +/- S.D. ; N=4

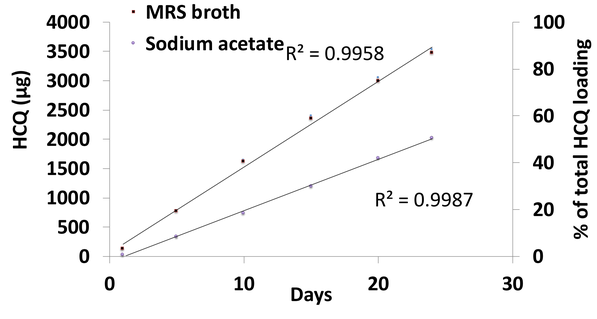

Fig 6: Cumulative release of HCQ from HCQ/K100M (ratio 1:1 wt/wt) IVR segments for 24 days. Data represents mean +/- S.D. ; N=4

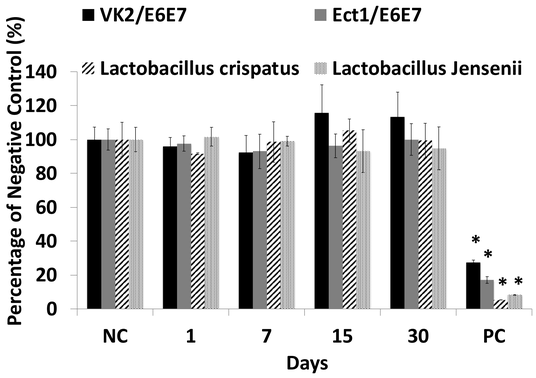

Fig 7: The impact of drug-free IVR segments on cell growth/viability. Data represents mean +/- S.D.; N=3; **P<0.05 vs NC*

Conclusion

IVR segment coated with a pH-sensitive polymer rapidly released fluorescent NP at pH8.2 and negligible amount at pH4.2. The reservoir-type IVR segment containing HCQ continuously released drug up to 21 days with a near zero-order release profile. The IVR segments did not present significant toxicity. The relative gene expression of CCR5 in cells treated with the siRNA-NP was significantly reduced compared to the non-treated cells.

Acknowledgments

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