Formulation of Stimuli-Sensitive Thiolated Hyaluronic Acid Based Nanofibers: Synthesis, Characterization, Preclinical Safety and In Vitro Anti-HIV Activity

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**Purpose**
To develop a bio-responsive, mucoadhesive hyaluronic acid-nanofibers (HA-SH-NFs) as a safe/effective carrier for the prevention of HIV virus vaginal transmission.

**Methods**
A novel sulfhydryl (-SH) group modified thiolated-HA was synthesized and characterized (FT-IR, NMR, XRD, and SEC analyses) to fabricate the TFV-loaded-HA-SH-NFs using electrospinning method. The surface morphology and size distribution of NFs were determined using scanning electron microscopy. The in vitro mucoadhesion of NFs was analyzed by ellipsometer and mucin-interaction assays. The in-vitro cytotoxicity of NFs was analyzed on cervicovaginal (CV) cells and Lactobacillus bacteria. In-vivo safety of NFs upon once-daily vaginal administration up to 7 days was assessed by histological analysis in female C57/BL6 mice. The inflammatory cells (CD45) infiltration in the genital tract up to 7 days of treatment of NFs was determined. The cytokine (IL-1α, IL-1β, IL-6, IP-10, IL-7, MKC, TNF-α) levels (pg/ml) in CV lavage and genital tissues were analyzed. The in-vitro anti-HIV activity of NFs was analyzed using pseudo type HIV virus-particles (mean diameter ~128nm, and titer ~3.07×1010 particles/ml) determined by nanoparticle tracking analysis. The anti-HIV activity was analyzed using a luciferase assay using different concentrations of free TFV and TFV loaded HA-SH-NFs.

**Results**
The sulfhydryl (-SH) group modified thiolated HA derivatives (HA-SH) were synthesized and characterized. The FT-IR and NMR analyses confirmed the effective thiol modification of native HA polymer. The SEC and PXRD analysis results confirmed that the native HA was stable under the thiol modification reaction process. The HA-SH based NFs were fabricated using electrospinning method and characterized for their surface morphology, and size distribution. SEM images showed the formation of HA-SH-NFs (mean diameter of ~75 nm). A higher mucoadhesion of NFs was observed compared to the native-HA based on an increase in the size (~4fold), thickness (~3fold) and adsorbed mucin amount (~2fold) after 3h incubation with mucin. A semen hyaluronidase enzyme triggered drug release (~87%w/w) from NFs occurred after 1h. The NFs were non-cytotoxic to CV cells and L. crispatus bacteria. Histological data showed no damage on the mice genital tract and other tissues on exposure to NFs. Following 24h of exposure, no significant CD45 cell-infiltration and the changes in cytokine levels were observed compared to control mice except for the IL-1α/TNF-α in genital track tissue and IL-1β/IP-10/TNF-α in CVL. However, the levels were well below than their standard values. The anti-HIV activity data suggested that the TFV-loaded-NFs were able to inhibit the virus replication and the structural-integrity and anti-HIV activity of TFV was unaffected by the electro-spinning process and composite geometry of NFs.

**Conclusion**
The data represented here highlight the potential of bio-responsive HA-SH-NF templates for the safety and vaginal delivery of anti-HIV/AIDS microbicides under the influence of hyaluronidase enzyme.