# Role of Microarchitecture in Co-delivery of Drug Combinations from Electrospun Fabrics

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**Statement of Purpose:** Combination drug delivery has several advantages compared to single-drug therapy in applications including cancer and HIV-1 therapy and antibiotic treatment[1]. However, there are many challenges associated with co-delivery of multiple drugs, including delivery of physicochemically diverse drugs and independently controlling the temporal release of the drugs[2]. Electrospun fibers are an elegant delivery vehicle for co-delivery as they are able to encapsulate a wide range of drugs and can be and can be independently tuned to realize a wide range of release kinetics[3,4]. We have developed a topical delivery system using electrospun fibers for multipurpose prevention of HIV-1 acquisition and unintended pregnancy using a combination of levonorgestrel (LNG), a hydrophobic contraceptive, and tenofovir (TFV), a hydrophilic antiretroviral. We aimed to investigate how the microarchitecture of the electrospun medical fabrics affected the fiber properties, *in vitro* release profiles, crystallinity of the drugs, and activity of encapsulated drugs.

**Methods:** Polyvinyl alcohol (PVA)-based electrospun fibers were prepared on an Elmarco NS 1WS500U. PVA solutions were loaded with 20 wt.% (wt. drug/wt. polymer) of LNG, TFV or both drugs. Three distinct composite microarchitectures were fabricated, including stacked, where layers of LNG and TFV fibers were consecutively spun, interwoven, where unique LNG and TFV fibers were spun at the same time, and combined, where LNG and TFV were loaded into the same solution and spun into the same fibers. Separate composite fabrics of all three microarchitectures were also spun with solutions co-loaded with fluorophores. We used scanning elctron microscopy (SEM) to characterize fiber size and morphology, differential scanning Calorimetry (DSC) to quantify the amount of crystalline drug in the fiber samples, and confocal microscopy to visualize the microarchitecture of fluorescent fibers. Release assays were performed at sink conditions in 1:1 isopropanol:water. Toxicity and HIV-1 BaL inhibition was measured in TZMbl cells.

**Results:** Irrespective of loaded drug (either LNG, TFV or both) and the microarchitecture, all electrospun fabrics were flexible, white, silky and macroscopically indistinguishable. The fibers showed a rounded, smooth morphology regardless of drug and loading up to 25 wt.%. All fibers were found to have < 2% crystalline TFV and LNG, and PVA fibers showed a slightly higher glass transition temperature when loaded with TFV. Confocal microscopy of fluorescent fibers revealed that the stacked fabrics had unique layers of LNG and TFV fibers, the interwoven fabrics had some unique but also some mixed TFV and LNG fibers, and the combined fabrics contained comingling TFV and LNG (Fig. 1a). *In vitro* LNG release was unaffected by combination with TFV, while TFV release was two-fold slower in the combined microarchitecture, but unaffected in the other composite microarchitectures. TFV release was also slowed with increasing fabric thickness, while there was no affect on LNG release. LNG was found to be toxic to TZMbl cells at concentrations of 5 μM and higher, while TFV was non-toxic at all tested concentrations. Inhibitory activity of TFV against HIV-1 BaL was maintained in all composite microarchitectures with an $IC_{50}$ of approximately 2 μM.

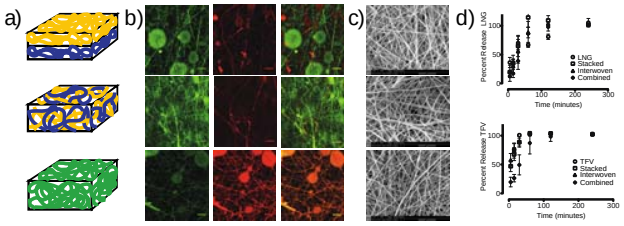


Figure 1. a) Illustrated schematic of stacked, interwoven and combined composite fibers (from top to bottom). b) Confocal images of fluorescently labeled composite microarchitectures. c) SEM micrographs of composite microarchitectures. d) LNG and TFV *in vitro* release from single drug and composite fabrics.

**Conclusions:** All fibers were prepared on a production-scale, free-surface electrospinning instrument with high productivity and encapsulation efficiency. Although the composite fabrics had distinct microarchitectures, there were no visual macroscopic differences. Only minimal drug crystallinity was detected, indicating that both LNG and TFV are mostly amorphous within the fibers. The shift in PVA glass transition temperature is likely due to hydrogen bonding interactions between the PVA and TFV. LNG release was unaffected by combination with TFV in any of the microarchitectures, as well as increasing the overall fabric thickness. However, TFV release was slowed when combined in the same fiber as LNG. TFV release was also considerably slower from the thicker fabrics. Reduction in TFV release from combined fibers may be attributed to the high LNG content (>10 wt.%), which likely dominates the dissolution process of these multicomponent materials. High concentrations of LNG caused cytotoxicity although these concentrations are much higher than physiologically relevant. TFV exhibited low cytotoxicity and potent activity against HIV-1 BaL infection *in vitro* for all the composite microarchitectures. Our results indicate the importance of microarchitecture on the design of electrospun fabrics for co-delivery of physicochemically diverse drugs. This system has potential to be developed as a topical, vaginal dosage for multipurpose prevention of unintended pregnancy and HIV-1 acquisition.

**References:** 1. Greco F. Advanced drug delivery reviews 2009;61(13):1203-1213. 2. Zhang L. ChemMedChem 2007;2(9):1268-1271. 3. Zhang Y. Biomacromolecules 2006;7(4):1049-1057. 4. Li X. Colloids and surfaces. B, Biointerfaces 2013;103:182-188.