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Supplementary Materials for

Drug-encapsulated carbon (DECON): A novel platform for enhanced drug delivery

Tejabhiram Yadavalli, Joshua Ames, Alex Agelidis, Rahul Suryawanshi, Dinesh Jaishankar, James Hopkins, Neel Thakkar, Lulia Koujah, Deepak Shukla*

*Corresponding author. Email: dshukla@uic.edu

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Supplementary Material

Supplementary Figures

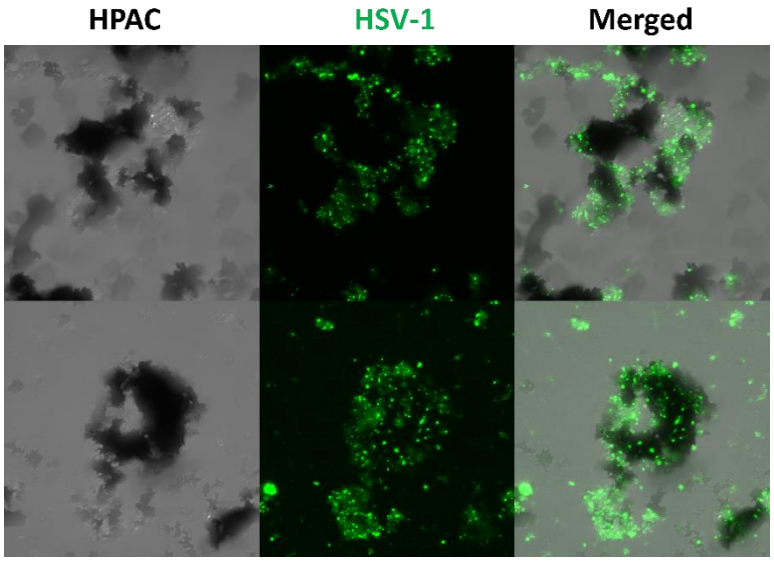

Fig. S1. HPAC strongly binds to HSV-1 GFP virus. 1 mg/mL HPAC was incubated with 106 PFU K26 GFP HSV-1 for a period of 20 minutes before the mixture was centrifuged at 10,000 g for 15 minutes. The pellet containing HPAC was washed multiple times with PBS before the mixture was suspended in fresh PBS. 10 µL of the mixture was dropped onto a glass slide and imaged at 100X on a confocal microscope.

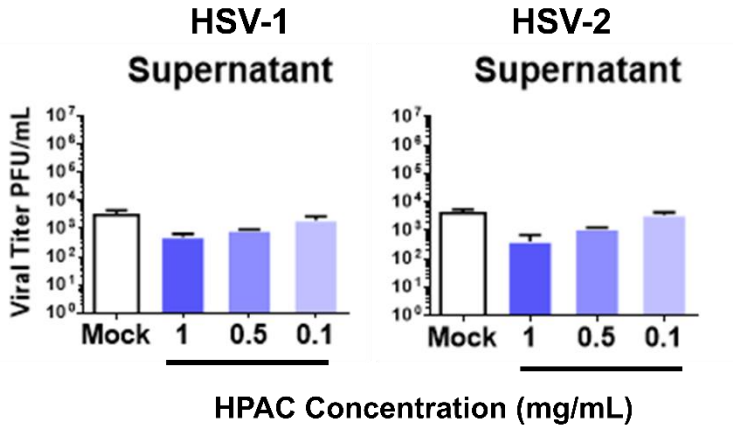

Fig. S2. Extracellular virus–based plaque assay for HPAC therapy. HCEs and HeLa cells were infected with HSV-1 or HSV-2 respectively prior to the therapeutic addition of mock (PBS) or HPAC. 24 hours post infection, supernatants from the samples were collected and overlaid on vero cells to perform a plaque assay.

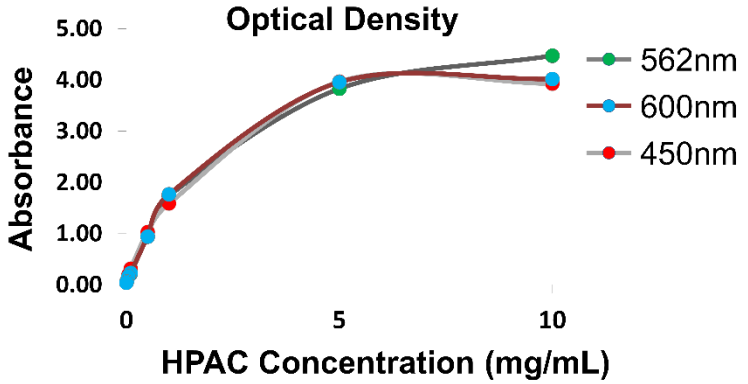

Fig. S3. HPAC does not cause a loss in visual acuity by blocking light. Various stock concentrations of HPAC were prepared in PBS and their optical density was measured using a standard plate reader at 450, 560 and 600 nm.

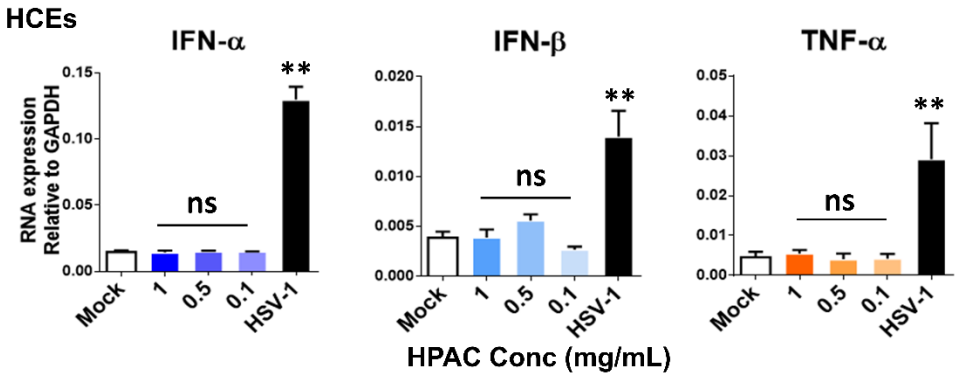

Fig. S4. HPAC does not elevate interferons in HCEs. Cells were either incubated with mock PBS (negative control), HPAC or HSV-1 (positive control). 24 hours post incubation, cells were lysed in TRIzol reagent and RNA was isolated using standard instructions. Isolated RNA was then reverse transcribed into cDNA using a reverse transcription kit. The cDNA was then analysed for the presence of the desired transcripts using a qRT-PCR machine.

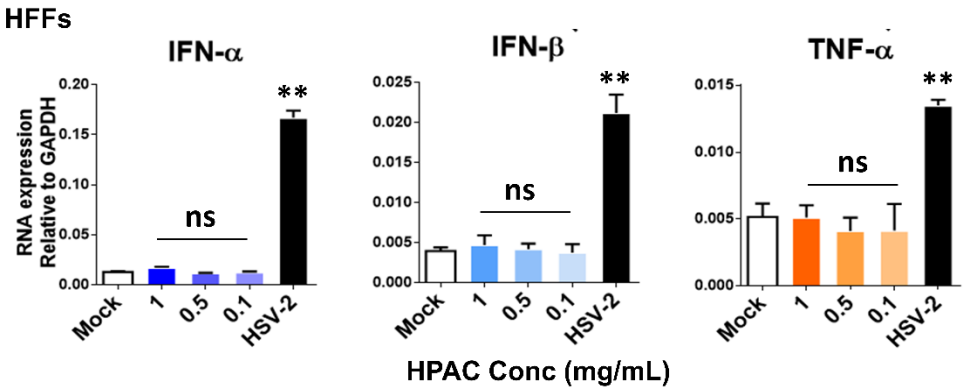

Fig. S5. HPAC does not elevate interferons in HFFs. Cells were either incubated with mock PBS (negative control), HPAC or HSV-2 (positive control). 24 hours post incubation, cells were lysed in TRIzol reagent and RNA was isolated using standard instructions. Isolated RNA was then reverse transcribed into cDNA using a reverse transcription kit. The cDNA was then analysed for the presence of the desired transcripts using a qRT-PCR machine.

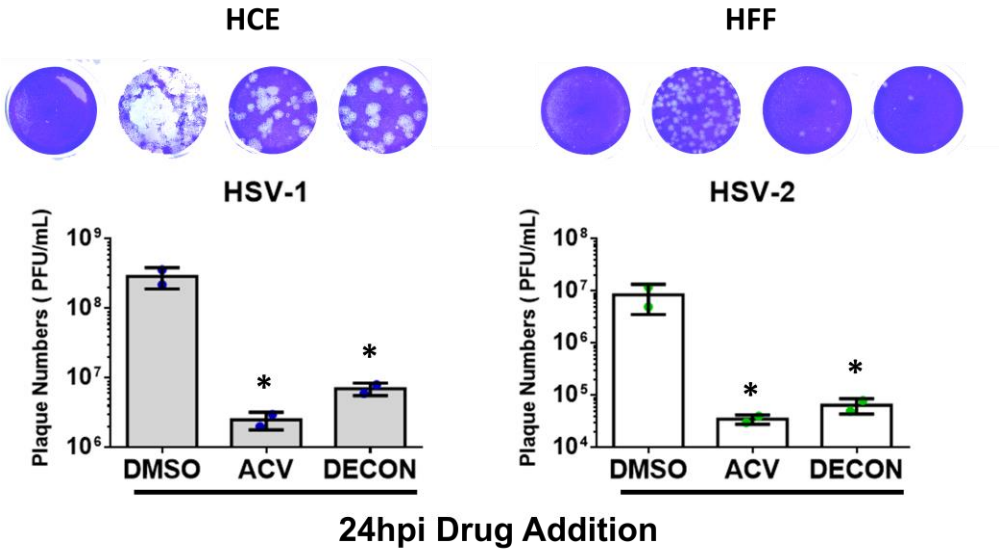

Fig. S6. DECON is effective when added 24 hours after infection. HCEs (Left) or HFFs (Right panel) were infected with HSV-1 or HSV-2 at an MOI of 0.1 respectively for a period of 24 hours. 24 hours post infection, mock DMSO, 50 µM ACV or 0.1 mg/mL DECON were added to the infected cells. 24 hours post drug addition, the cells were collected, lysed and overlaid on Vero cells to conduct a plaque assay.

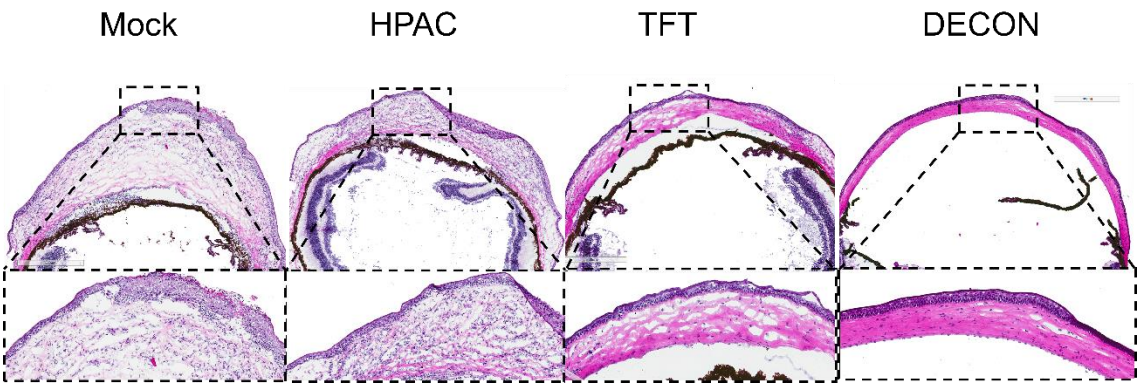

Fig. S7. DECON protects the murine cornea from HSV-1 infection. Mice were infected with HSV-1 after corneal debridement. Treatments were started on day 1 post infection. Murine eyes collected on day 21 were frozen in OCT medium and 10 µM ocular sections were stained with H&E stain. Top images were taken at 2.5x magnification and bottom bars are 20x magnifications of the same image.

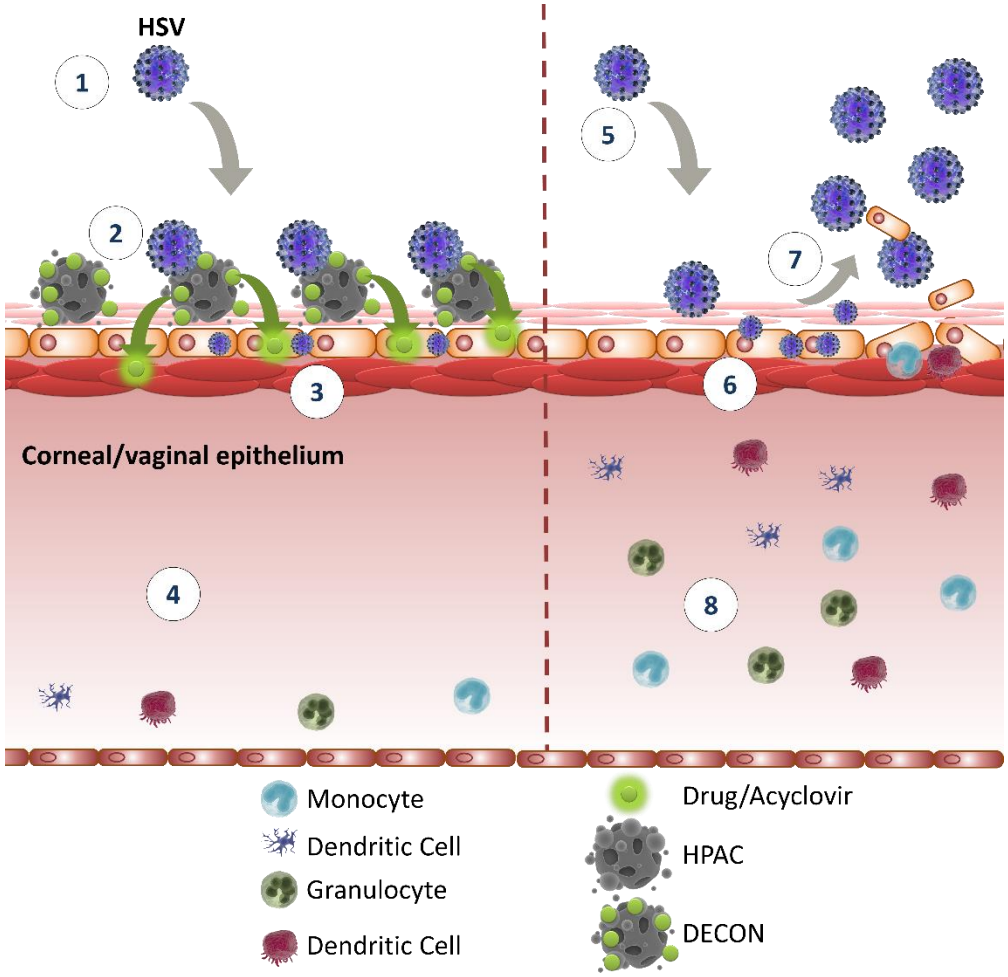

Fig. S8. Graphical abstract showing DECON protecting cells against viral infection. When cells are treated with DECON particles during HSV infection, DECON binds to the surface of the cells and is protected from rapid clearance in the corneal and vaginal epithelium. While on the surface, DECON traps incoming viruses. The act of trapping the virus ensues the release of ACV in to the surrounding cells resulting in their protection from viral infection. While in non-treated or topically treated (with non-DECON drugs) cells, there is no deterrent to the incoming virus which results in infection, replication and subsequent inflammation of the corneal or vaginal epithelium. Given that HPAC and DECON do not entail an immune response by themselves; they will be safe to use in ocular drops or genital ointments.

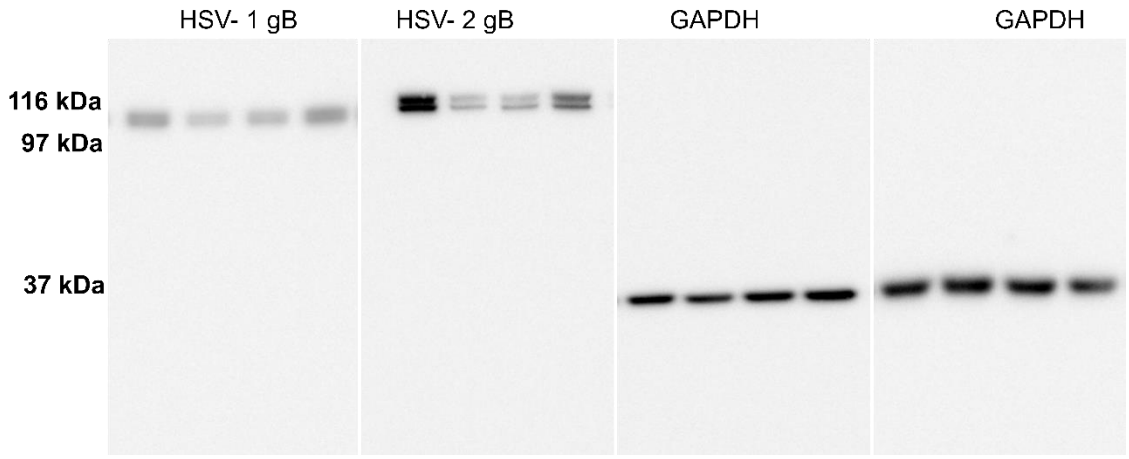

Fig. S9. Full-length blots for the Western blot shown in Fig. 1 (G and H).

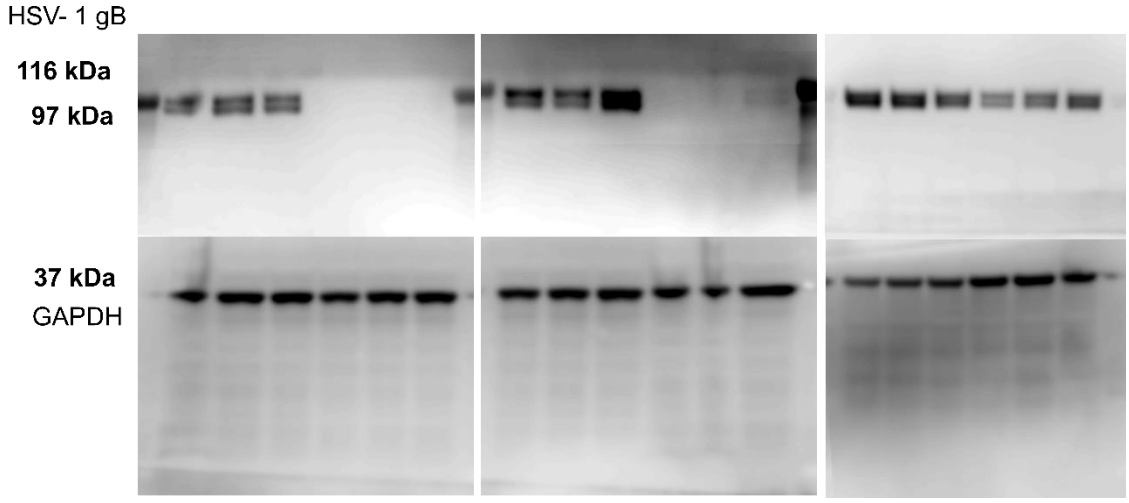

Fig. S10. Full-length blots for the Western blot shown in Fig. 3D.