Nanoparticle-mediated E-selectin siRNA Delivery Reduces Inflammation in MHV-3 Infected Mice

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BACKGROUNG

- Murine hepatitis virus 3 (MHV-3) induces a dysregulated immune response (cytokine storm).
- Targeting E-selectin, which mediates leukocyte recruitment to inflamed tissues, offers a potential strategy to mitigate liver inflammation, reduce hepatocyte death, and alleviate microvascular thrombosis.
- The delivery of siRNA in vivo remains a major challenge due its instability in the bloodstream, immunogenicity, and difficulty in crossing biological barriers.

OBJECTIVES

- Prepare E-selectin and AF647 siRNA-loaded HNPs.
- Investigate formulation physicochemical attributes.
- Test the uptake in vitro and biodistribution in vivo.
- Assess whether silencing with HNP-siEsel will be able to modulate the inflammatory response after MHV-3 infection.

METHODOLOGY and RESULTS

a) Development and characterization of HNP-siAF647 and HNP-siEsel

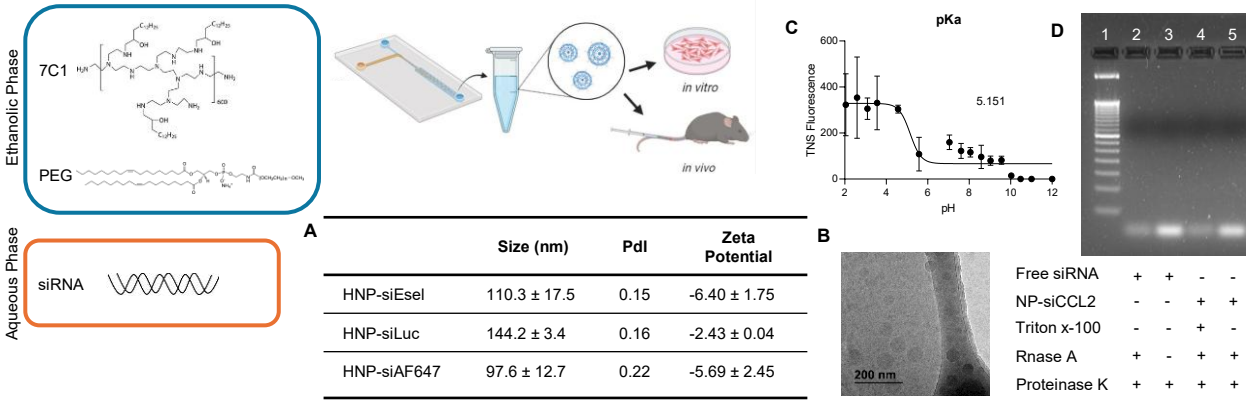

Figure 1. Schematic ilustration of the NPs preparation by microfluidic mixing. (A) Physicochemical caracterization of the HNPs. (B) Cryo-TEM of the HNP-siEsel. (C) pKa determination by TNS assay. (D) Rnase protection assay.

b) In vitro uptake and in vivo biodistribution of HNP-siAF647 is time dependent

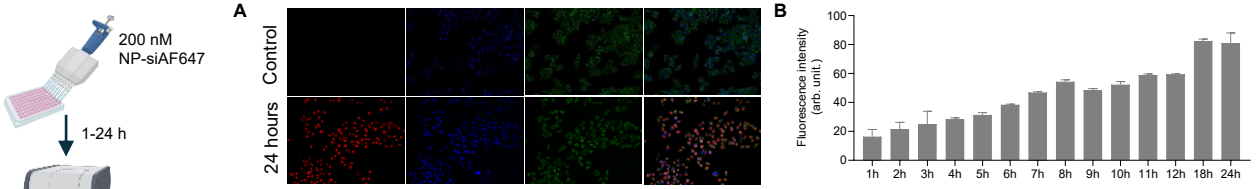

Figure 2 – **(A)** Fluorescence photomicrographs of in vitro uptake and **(B)** quantification of NP-siAF647 in HepG2 cells after treatment with siRNA (200 nm) for 1-24 hours. NPs are labeled with siRNA fluorescent (red), the nuclei are stained with DAPI (blue), and the citoeskeleto are stained with Alpha-actinin (green). Data are shown as mean ± standard deviation and analyzed by one-way ANOVA of multiple comparisons. **p<0.01; ***p<0.001

METHODOLOGY and RESULTS

c) In vivo biodistribution of NP-siAF647 is time dependent

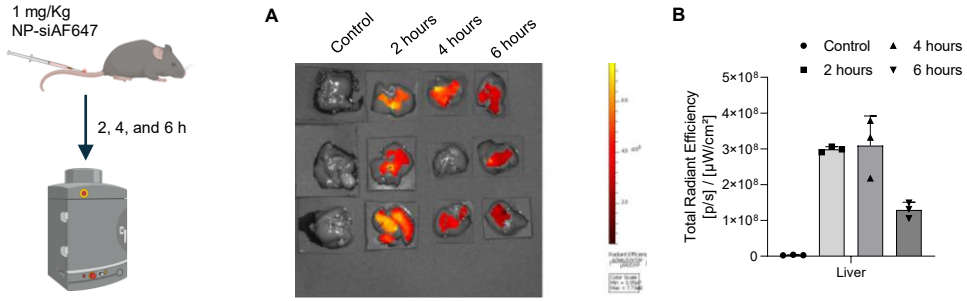

Figure 3. **(A)** NP-siAF647 biodistribution in healthy C57BL/6 mice 2, 4, and 6 hours after intravenous administration (IV). **(B)** Fluorescence quantification in liver 2, 4, and 4 hours after IV injection of NP-siAF647. Data are shown as mean ± standard deviation and analyzed by one-way ANOVA of multiple comparisons. **p<0.01; ***p<0.001

d) HNP-siEsel reduce viral load and pro-inflammatory cytokines in liver

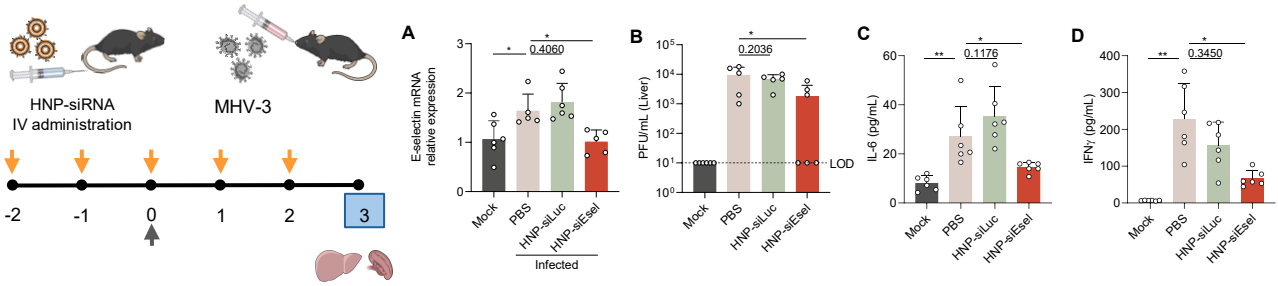

Figure 4. Overview of treatment (1 mg/Kg; everyday) and infection (1x10⁴). **(A)** Gene silencing after treatment with HNP-siEsel measured by RT-qPCR. Interest gene was normalized by GAPDH. **(B)** Viral titer in liver determined by plaque assay. LOD: limit of detection. **(C-D)** Cytokynes measured by CBA Inflammatory kit. Data are shown as mean ± standard deviation and analyzed by one-way ANOVA of multiple comparisons. *p<0.05 **p<0.01; ****p<0.0001

e) HNP-siEsel reduce myeloid cells population in liver

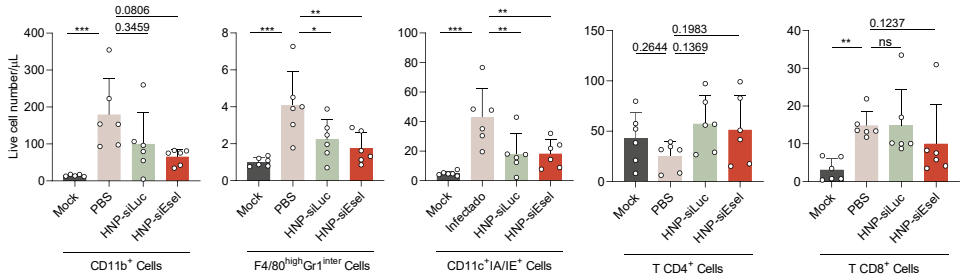

Figure 5. (A) Liver myeloide cells profile at 3 DPI for each group. E-selectin silencing reduced the monocytes (B) and dendritic cells (C) in liver. No alteration in lymphocyte population was observed after HNP-siRNA treatment even in T CD4+ (D) or T CD8+ (E). Data are shown as mean ± standard deviation and analyzed by one-way ANOVA of multiple comparisons. *p<0.05 **p<0.01; ***p<0.001

CONCLUSIONS and PERSPECTIVES

We have developed a hybrid lipid-polymer HNP for siRNA delivery which induced the silencing of E-selectin in the liver tissue. Treatment with HNP-Esel reduced viral load in the liver of mice infected with MHV-3. We also found reduced pro-inflammatory cytokines (IL-6 and IFNγ). In conclusion, this strategy highlights the potential of modulating immune responses to improve disease outcomes in viral infections, providing a rational approach for further investigation into the role of inflammation in MHV-3 pathogenesis.

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