Advanced Nanoparticle Design for HSV-1 Dormant State Targeting

Objective

The nanoparticle is designed to remain dormant in a host's body until it detects reactivation of HSV-1. Upon reactivation, the nanoparticle delivers antiviral drugs or gene-editing tools to suppress viral replication, potentially reducing the viral reservoir over time. This nanoparticle design focuses on efficiency, specificity, and safety.

Core Nanoparticle Material

The core of the nanoparticle is made from a **PLGA (Poly(lactic-co-glycolic acid)) matrix**, selected for its biocompatibility, biodegradability, and FDA-approved status for use in drug delivery systems. PLGA degrades into lactic acid and glycolic acid, which are safely metabolized by the body.

- **PLGA Molecular Weight:** 50:50 lactic acid:glycolic acid ratio for a balanced degradation time (weeks to months).

- **Particle Size:** 80-100 nm, optimized for:
  - Enhanced permeation and retention (EPR) effect for inflamed or infected tissues.
  - Efficient intracellular uptake through endocytosis by infected nerve cells.

Surface Functionalization

1. PEGylation for Circulation Time
To ensure the nanoparticles evade the immune system and remain dormant in the bloodstream for extended periods, the surface is coated with **polyethylene glycol (PEG).**

  - **PEG Molecular Weight:** 5 kDa (small enough to avoid immune detection but large enough to prevent aggregation).

  - **Conjugation Method:** Carbodiimide chemistry (EDC/NHS) is used to attach PEG to the surface of the PLGA core via covalent bonds.

2. Targeting Ligands
  - **HSV-1 Glycoprotein gD Targeting:** Nanoparticles are surface-functionalized with monoclonal

antibodies or peptide ligands specific to **HSV-1 glycoprotein D (gD)**, which is expressed on infected cells upon reactivation.

- **Peptide Design:** A 12-mer peptide derived from the viral entry mechanism is conjugated to the nanoparticle surface using maleimide chemistry, allowing it to selectively bind to HSV-1-infected cells.

3. Enzyme-Sensitive Linker

- **Protease-Sensitive Coating:** The outer layer of the nanoparticle is coated with an enzyme-sensitive polymer that is cleaved by viral proteases (e.g., ICP0 protease) during HSV-1 reactivation. This ensures that the nanoparticles remain dormant until viral protease activity is detected, triggering payload release.

Cargo (Therapeutic Agents)

1. Antiviral Drug Payload

Encapsulated Antiviral Agent: The nanoparticles encapsulate **valacyclovir**, a prodrug of acyclovir, known for its ability to inhibit HSV-1 DNA polymerase, effectively preventing viral replication.

- **Encapsulation Efficiency:** Achieved using a double emulsion solvent evaporation method, with drug loading optimized at **10-15% w/w**.

2. Gene Editing Cargo (Optional)

In addition to antivirals, the nanoparticles can encapsulate CRISPR-Cas9 machinery targeted at HSV-1's latent viral genome:

- **Cas9 Nuclease:** Encapsulated Cas9 protein with a guide RNA targeting **ICP4** or **LAT (Latency-Associated Transcript)** regions in the HSV-1 genome.
- **Delivery Vector:** The CRISPR-Cas9 system is encapsulated in **cationic lipid nanoparticles** within the PLGA core, ensuring efficient delivery to infected cells.
- **Goal:** Permanent disruption of the viral genome in latently infected cells, leading to the potential eradication of the latent reservoir.

Synthesis Protocol

1. Nanoparticle Fabrication

Method: Double emulsion solvent evaporation technique.

- **Step 1:** Dissolve PLGA in dichloromethane to form the oil phase.

- **Step 2:** Add valacyclovir dissolved in water to form the primary emulsion (W/O). Sonicate for 2 minutes.

- **Step 3:** Add the primary emulsion to a polyvinyl alcohol (PVA) solution to form a W/O/W emulsion. Sonicate again for 3 minutes.

- **Step 4:** Evaporate the solvent under reduced pressure, allowing PLGA nanoparticles to form.

- **Step 5:** Add PEG and ligands through carbodiimide-mediated reactions (EDC/NHS), then purify using centrifugation and filtration.

2. Surface Functionalization with Targeting Ligands

- **Peptide Conjugation:** The targeting peptide for HSV-1 glycoprotein D is conjugated using maleimide chemistry. This creates a stable thiol-maleimide bond between cysteine residues on the peptide and maleimide groups on the nanoparticle surface.

Mechanism of Action

1. Dormancy State

- **PEGylation and Protease-Responsive Layer:** Nanoparticles circulate in the body in a dormant state, protected by PEGylation and a viral protease-sensitive outer coating. The nanoparticles do not interact with healthy cells and remain stable at physiological pH (7.4).

2. Trigger for Activation

- **Protease Cleavage:** Upon HSV-1 reactivation, the viral protease ICP0 cleaves the outer enzyme-sensitive coating of the nanoparticle.

- **Exposure of Targeting Ligands:** This exposes the gD-targeting ligands, which bind specifically to HSV-1-infected cells expressing glycoprotein D.

- **Payload Release:** Once bound to the target cells, the nanoparticle releases its cargo (valacyclovir or CRISPR-Cas9) via diffusion, directly inhibiting viral replication or permanently disrupting the viral genome.

3. Selective Activation

- **Staggered Activation Mechanism:** Only nanoparticles in the immediate vicinity of reactivated HSV-1 will activate, ensuring efficient and localized drug delivery. This prevents widespread activation and conserves the nanoparticle supply for multiple reactivation events.

Characterization

1. Particle Size and Distribution

- **Dynamic Light Scattering (DLS):** Used to measure the average particle size (80-100 nm) and polydispersity index (PDI < 0.2).

- **Transmission Electron Microscopy (TEM):** Used to confirm the spherical morphology and structural integrity of the nanoparticles.

2. Surface Charge

- **Zeta Potential:** Measured to ensure the nanoparticles have a slight negative charge (-10 to -20 mV), indicating good colloidal stability in biological fluids.

3. Drug Loading Efficiency

- **UV-Vis Spectroscopy:** Used to quantify the amount of encapsulated valacyclovir, ensuring drug loading efficiency of 10-15%.

4. In Vitro Release Profile

- **Dialysis Method:** Performed to test the release kinetics of the antiviral drug in a controlled pH environment (pH 5-6 simulating reactivation conditions). The release should follow a sustained profile, with >70% of the drug released over 24 hours.

In Vivo Testing

1. Animal Model

- **HSV-1 Latency Model in Mice:** Mice latently infected with HSV-1 are used to test nanoparticle efficacy. The nanoparticles are administered intravenously, and viral reactivation is triggered using heat stress or immunosuppression.

2. Efficacy Testing

- **Viral Load Reduction:** PCR and viral plaque assays are used to measure viral DNA and active

virus in tissues after nanoparticle treatment.

- **Latency Clearance:** After repeated treatments, the latent viral reservoir is measured to assess whether the CRISPR-Cas9 system has effectively excised the viral genome.

3. Safety and Toxicity

- **Biocompatibility:** Hemolysis assays, liver/kidney function tests, and histopathological analysis are performed to assess nanoparticle safety and potential off-target effects.

Conclusion

This advanced nanoparticle design offers a targeted, efficient approach to treating HSV-1 reactivation. By remaining dormant until viral reactivation is detected and delivering precise antiviral or gene-editing agents, the nanoparticles could significantly reduce viral reactivation events and, over time, eliminate the latent viral reservoir.