PLGA Nanobots for therapeutic siRNA delivery: A novel strategy for bladder cancer therapy

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ABSTRACT

- ✓ **Bladder cancer** (BC) is a significant global health concern, ranking as the 9th most common cancer worldwide and the 2nd most prevalent genitourinary malignant disease with a 70% relapse rate [1].
- ✓ The use of urease-powered **nanobots (NBs)** is an innovative therapeutic approach due to:
  - Fuel-induced collective displacement.
  - Use of physiologically an available fuel
  - *In vivo* penetrance and distribution in the tumor [2].
- ✓ **Short interference RNA (siRNA)** can be used to specifically and efficiently knock-down target gene expression using cellular machinery (Figure 1) [3].

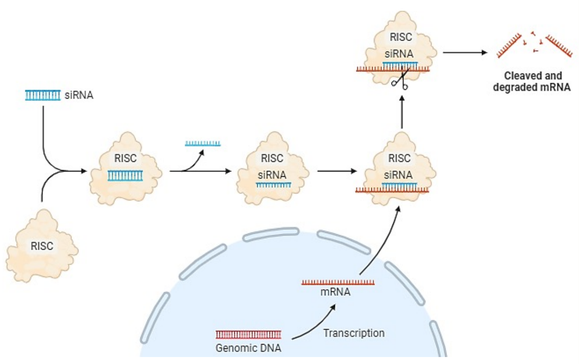

Figure 1. Mechanism of siRNA-induced gene expression knock-down.

RESULTS

Nanoparticles (NPs) were synthesized using biocompatible/biodegradable poly (lactic-co-glycolic acid) (**PLGA**), an FDA/EMA approved scaffold [4]. Using a layer-by-layer (LBL) approach, siRNA was loaded onto the NBs as shown in Figure 2A. Urease-functionalized NBs, were analyzed by dynamic light scattering (DLS), to determine size (nm), zeta-potential and polydispersity index (PDI) (Figure 2B). Urease attachment was quantified by BCA, reaching 39% (Figure 2C).

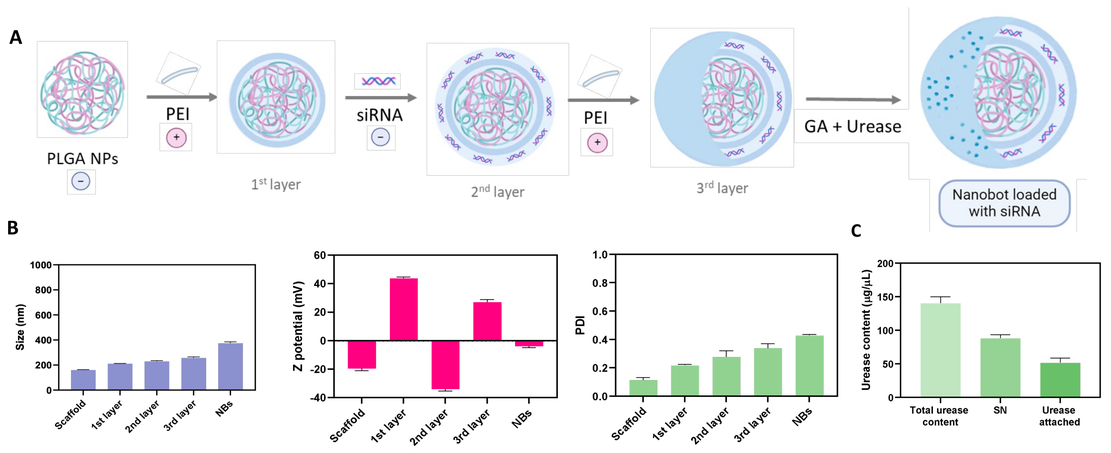

Figure 2. A. LBL synthesis of siRNA-loaded NBs representation. **B**. DLS analysis of size, zeta potential and PDI of PLGA NBs. **C.** BCA analysis for the quantification of urease attached to the NBs.

The optimal loading of siRNA was achieved with a capacity of 67.5 µg of siRNA/mg of PLGA NPs. Afterwards, siRNA loading, and subsequent release was assessed by incubating the NPs with sodium dodecyl sulfate (SDS) (Figure 3).

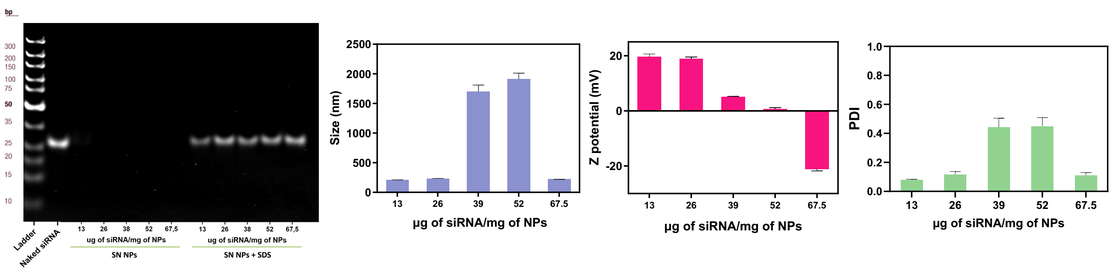

Figure 3. A. Polyacrylamide gel electrophoresis analysis of siRNA content in supernatant (SN) and NPs loaded with different ratios of siRNA. **B.** DLS data of the NPs with the different ratios of siRNA.

NBs motion analysis was performed by measuring the covered area in the presence or absence of urea as a substrate. NBs increased the percentage of area covered as a function of urea concentration (Figure 4A and B). Also, NBs siRNA release study shows that in the presence urease substrate, siRNA is released after 1h (Figure 4C).

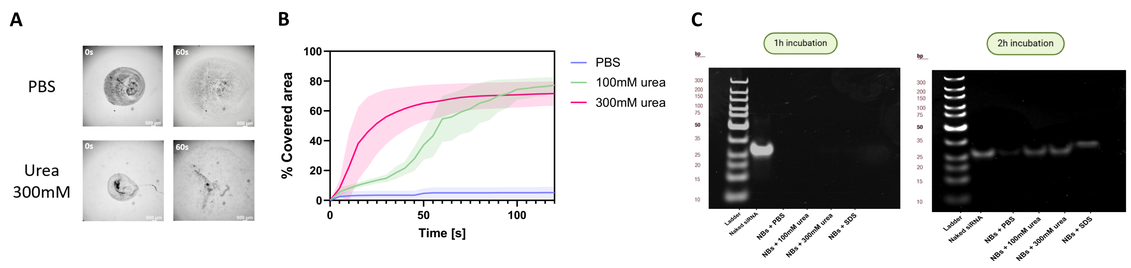

Figure 4. A. Frames of motion videos of the NBs at the indicated time points. **B.** Plot of the area covered by the NBs. **C.** Release study of siRNA NBs incubated with urease substrate at 1h/2h timepoints, respectively.

To test the delivery efficiency of the cargo, endosomal escape capacity of the different polymers was tested with NPs loaded with an AF647-conjugated oligonucleotide (Figure 5A) and analyzed after 24h by confocal microscopy in murine MB49 cell line (Figure 5B). Similar results were achieved in human RT4 spheroids.

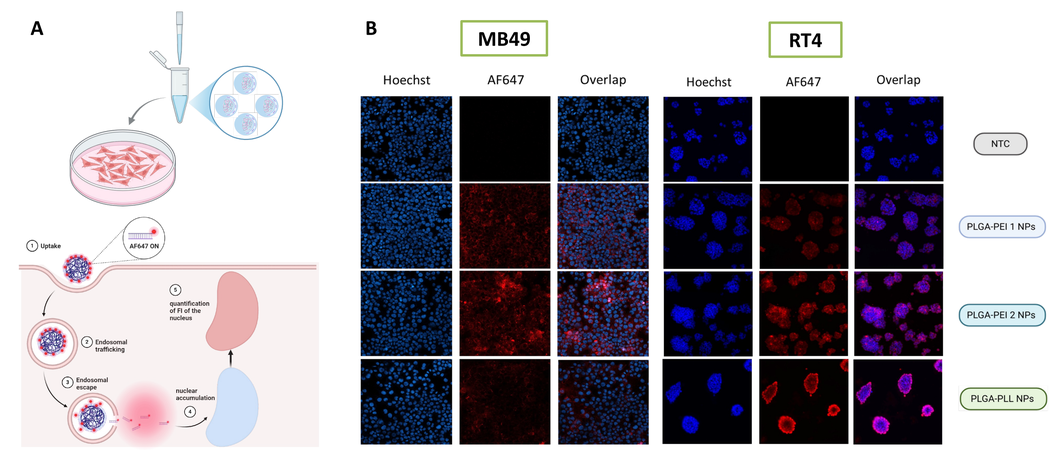

Figure 5. A. Strategy to analyze endosomal escape capacity of each formulation. **B.** Endosomal escape of the indicated formulations analyzed by confocal microscopy.

Knock-down efficiency of the different NPs formulations loaded with siRNA against GFP or control as indicated, was analyzed by flow cytometry after 72h incubation with H1299 cells, that express GFP constitutively (Figure 6).

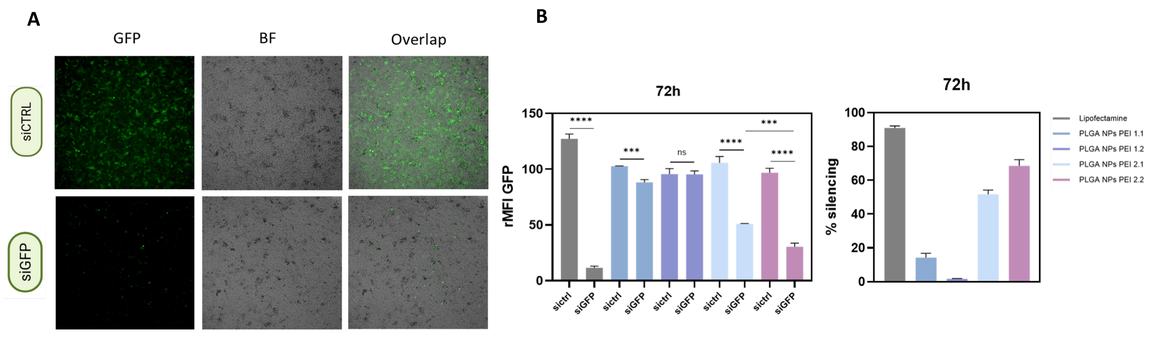

Figure 6. A. GFP knock-down by PLGA NPs PEI 2.2 shown by fluorescence microscopy.
B. rMFI of GFP at 72h post-incubation with the indicated formulations of PLGA NPs and (left panel) and percentage of GFP silencing of the different formulations (right panel).

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CONCLUSIONS

- The loading of siRNA into the NBs was successfully achieved.
- The NBs were synthetized, characterized and move in the presence of the fuel, urea.
- The polymers used in the synthesis of the NPs play a critical roll in the endosomal escape, to deliver efficiently the siRNA.

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