Dual-Modality Poly-L-histidine Nanoparticles to Deliver Peptide Nucleic Acids and Paclitaxel for In Vivo Cancer Therapy

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Purpose:

The popularity of cationic polymeric nanoformulations for improving the transfection efficiency is well known. However, the excess of positive charge limits their clinical transition as it often leads to severe cell and tissue-based toxicity. Due to their biocompatibility and natural origin, cationic amino acids-based polymers offer several advantages as compared to synthetic polymers. Although, histidine based branched copolymers have been explored earlier, their rigorous synthesis and scale-up challenges limit their clinical translation. Herein, we investigated a series of cationic poly-(lactic-co-glycolic acid) (PLGA)-histidine based nanoformulations which are simple, easy to scale-up and whose surface charge could be fine-tuned for enhanced cytoplasmic delivery with minimal toxicity. We tested PLGA-histidine formulations for delivery of small molecule and nucleic acid based (peptide nucleic acid targeting microRNA-155) drugs.

Methods:

We developed a series of PLGA-histidine formulations using double emulsion solvent evaporation technique for the delivery of small molecule and peptide nucleic acids. Different solvent mixtures were explored for improved morphology, uniformity, and size. Furthermore,

comprehensive physico-biochemical characterizations of histidine-based formulations including loading analysis, release kinetics and cellular uptake studies using confocal and flow cytometry-based methods were conducted. Using different endocytosis inhibitors, cellular uptake mechanisms of these formulations were studied. Small-angle neutron scattering (SANS) experiments were conducted to deduce the structural arrangement of histidine in these PLGA formulations. For proof of principle, Paclitaxel, and peptide nucleic acids (PNA) targeting microRNA-155 were encapsulated in PLGA-poly-L-histidine formulations and tested *in vitro* followed by extensive *in vivo* efficacy studies in xenograft mice model. Endpoints of tumor growth inhibition and immunohistology were evaluated. For PNA-155 treated group, microRNA-155 levels and downstream targets were also evaluated. In addition, safety of PLGA-poly-L-histidine nanoformulations was established by histology, blood chemistry and complete blood count analysis.

Results:

We have successfully developed and optimized PLGA-histidine formulation with optimum solvent conditions that generated polymeric nanoparticles of small particle size (~170nm hydrodynamic diameter) with remarkable polydispersity index. Furthermore, using different weight ratios of PLGA and poly-L-histidine, optimum surface charge could be achieved for these formulations without compromising on their cellular uptake properties. Overall, the lead formulation of PLGA-poly-L-histidine showcased highest cellular uptake by clathirin-mediated endocytosis than other PLGA formulations. The dry particle size and spherical morphology of PLGA-poly-L-histidine nanoformulations was confirmed by SEM and TEM imaging. Further structural evaluations by SANS experiments confirmed the presence of small poly-L-histidine patches of 1 nm in size along the surface of the PLGA nanoparticles. Furthermore, PLGA-poly-L-histidine showed superior inhibition of tumor growth in PNA-155 (~6 fold) and paclitaxel (~6.5 fold) treatment groups in comparison to PLGA *in vivo.* PNA-155 PLGA-poly-L-histidine formulation exhibited a significant decrease in microRNA-155 levels up to (~45%) in comparison to the control group. Similarly, PNA-155 PLGA-poly-L-histidine treated group showed upregulation of downstream targets of microRNA-155 such as Foxo3A (~1.3 fold) and Bach1 (~1.2 fold) in comparison to the control. Immunohistochemistry analysis by Ki-67 staining indicated lower proliferation in PLGA-poly-L-histidine treated tumors with minimal toxicity. The safety evaluation of positively charged PLGA-poly-L-histidine formulations by blood chemistry, complete blood count (CBC), and organ histological analysis indicated no toxic effects in both PNA-155 and Paclitaxel treatment group in comparison to the control group.

Conclusions:

We have demonstrated an easy, scalable poly-L-histidine containing PLGA nanoformulations with superior cellular distribution and *in vivo* efficacy compared to other PLGA formulations. We established that aforementioned formulation can effectively deliver small molecules

(chemotherapeutics) and nucleic acid analogs (PNA) for various therapeutic applications in a safe manner.

Keywords: (Poly-L-Histidine, PLGA, nanoparticles, endosomal escape, microRNAs)

Figures:

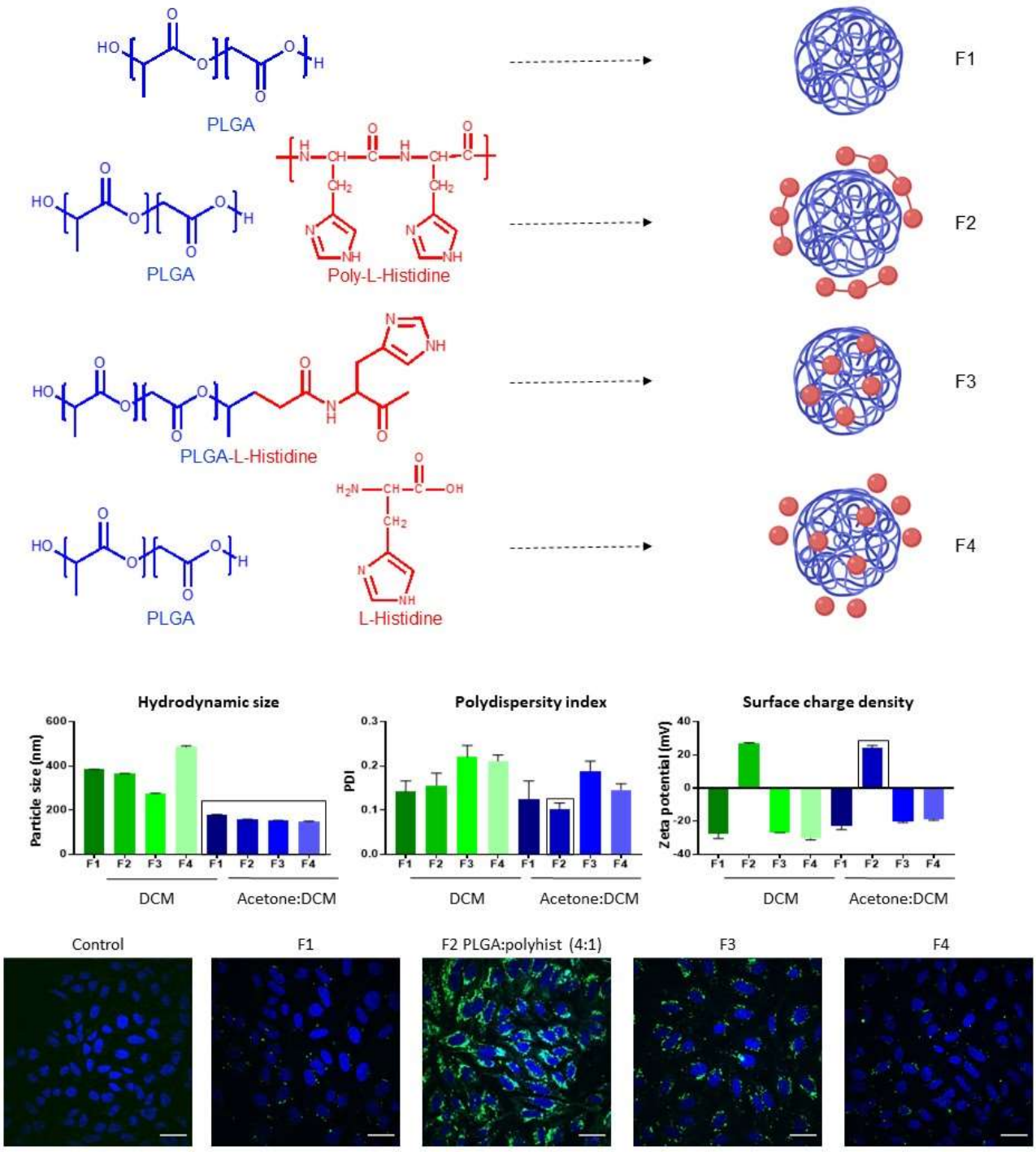

Figure 1. Schematic showing (A) Chemical structure of PLGA and histidine components and their representative arrangement in nanoparticles. (B) Characterization of nanoparticle formulations. (C) Cellular uptake in HeLa cells

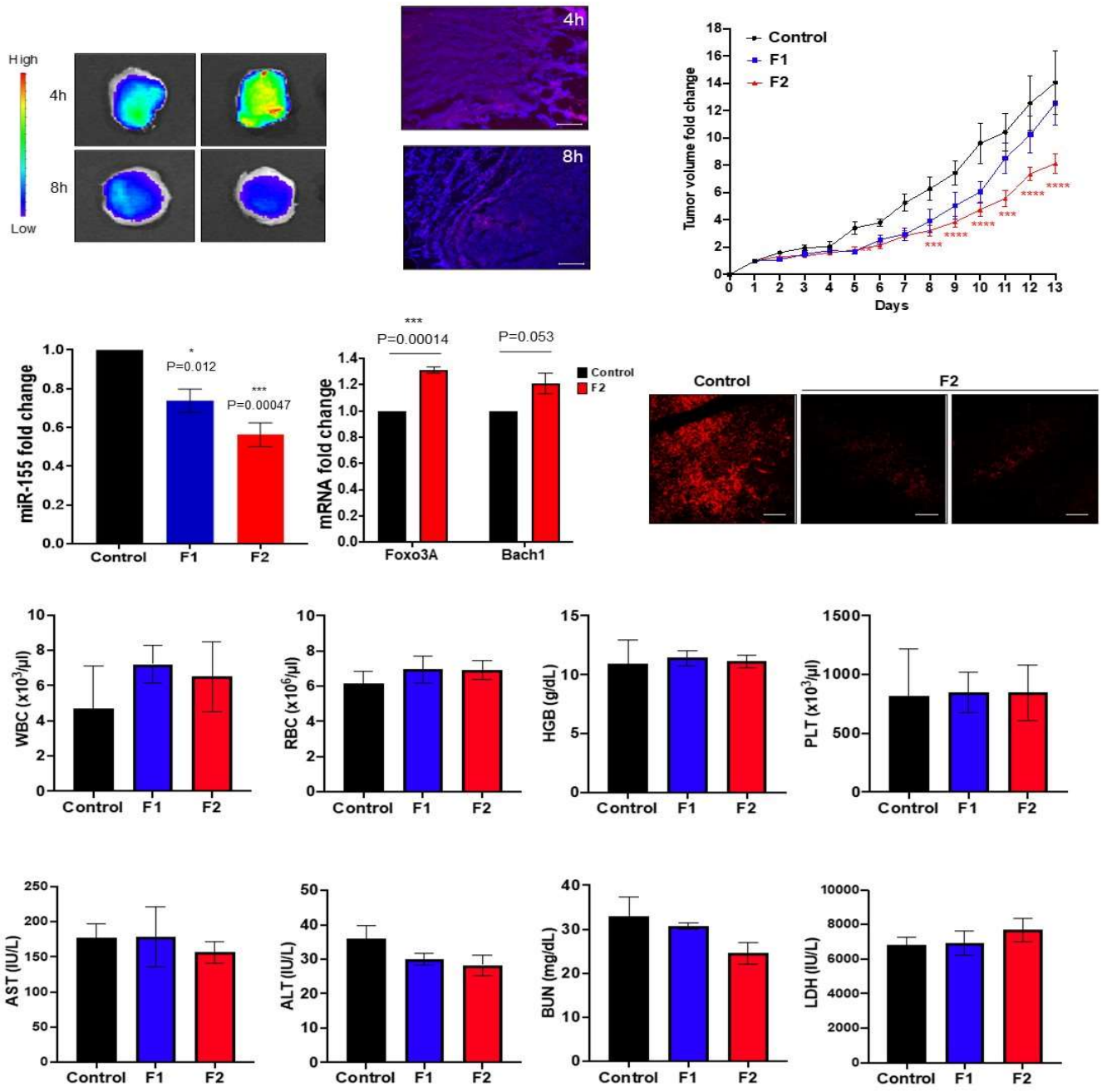

Figure 2. (A) & (B) Tumor accumulation of nile red F2 NPs after 4 and 8 hours (C) The tumor volume fold change curve after systemic treatment with PNA-155 loaded F1 and F2 NPs. (D) & (E) The relative expression levels of miR-155 and Foxo3A and Bach1 (miR-155 downstream targets). (F) Cell proliferation marker Ki67 immunostaining of tumor section from control and two different F2 NPs treated tumors. (G) &(H) The clinical chemistry & blood biochemistry of F1 and F2 NPs treated xenograft mice.

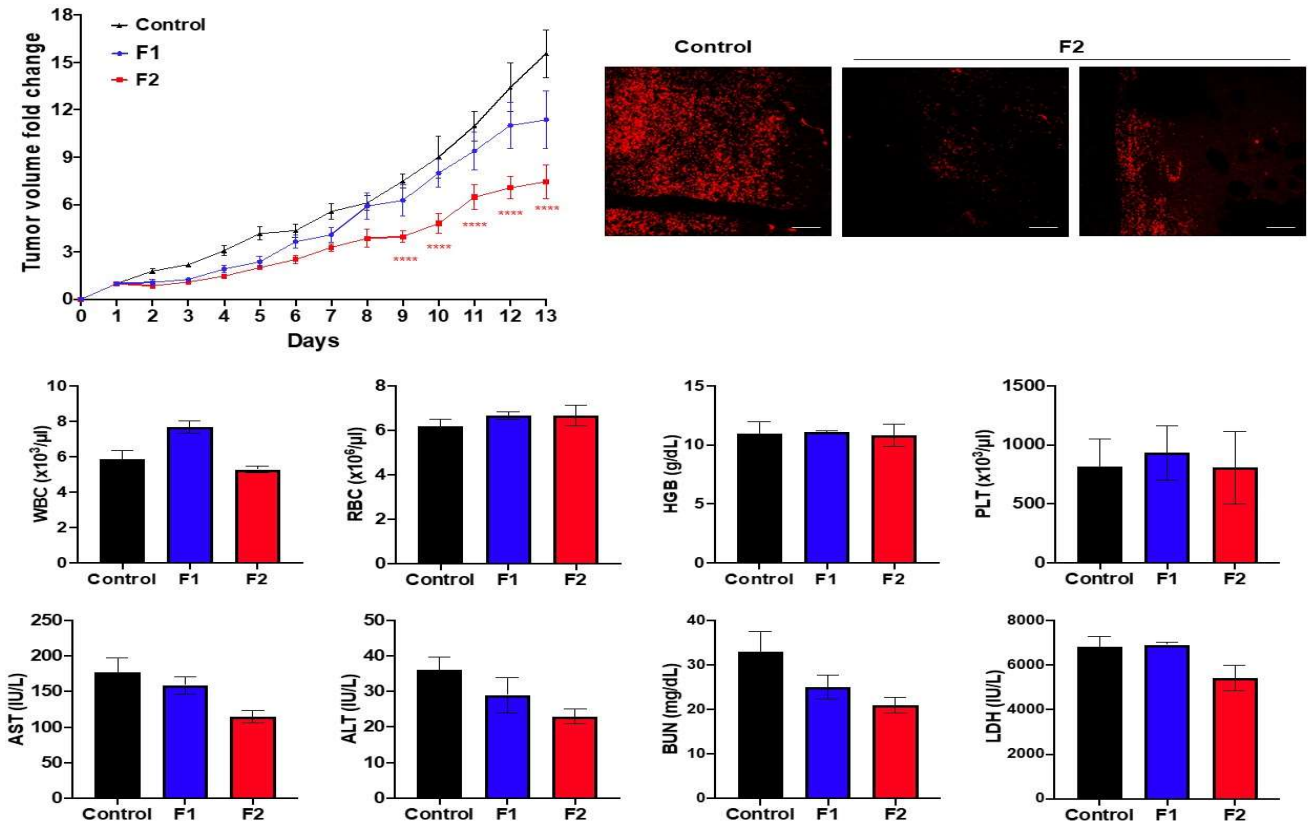

Figure 3. (A) The tumor growth fold change curves after systemic treatment with paclitaxel loaded F1 and F2 NPs. (B) Cell proliferation marker Ki67 staining of tumor section from control and two different F2 NPs treated tumors. (C) The clinical chemistry & blood biochemistry of Paclitaxel loaded F1 and F2 NPs treated xenograft mice.