THE UNIVERSITY OF CHICAGO
PRITZKER SCHOOL OF MOLECULAR ENGINEERING

HA-PEG Layered F-PEI Core-Shell Micelles for PDGFRβ Targeted Delivery of TXNDC5 Silencing for Pulmonary Fibrosis

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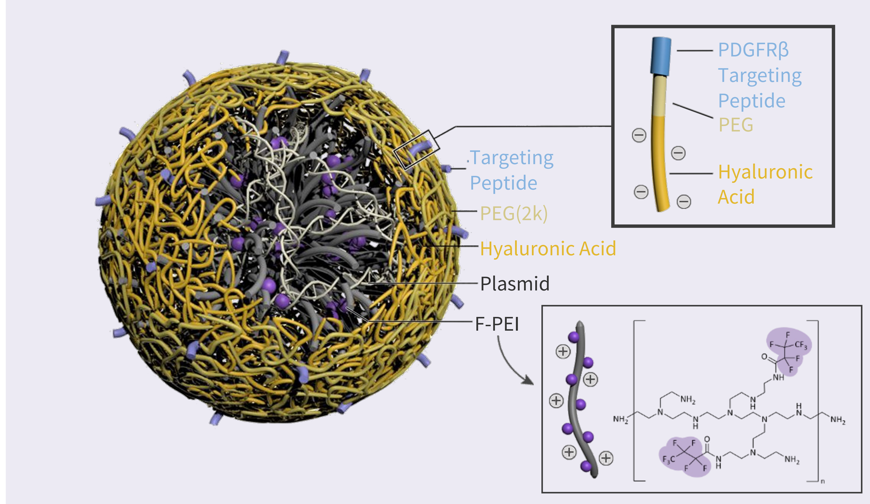
Highlights

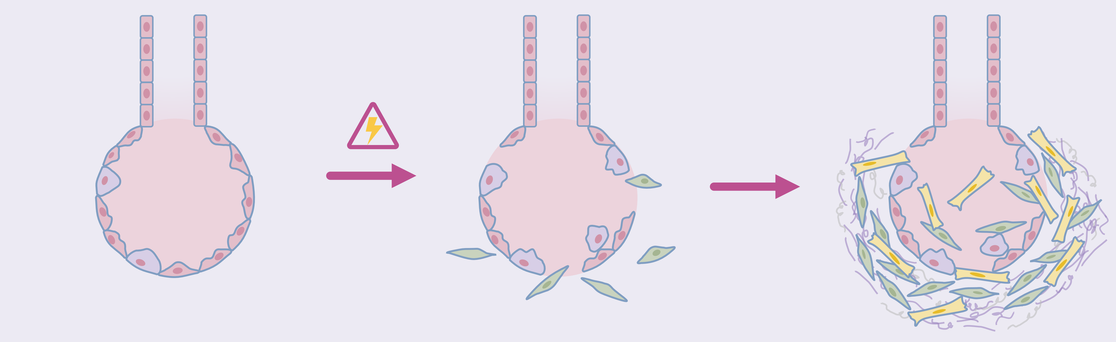
- Multifunctional core–shell design enhances stability, circulation, and targeted shRNA delivery
- F-PEI self-assembles with shRNA to form compact, fully condensed cores
- PDGFRβ-targeted delivery improves uptake in fibrotic lung cells
- HA-PEG shelling retains endosomal escape and ensures high cell viability
- Therapeutic efficacy in bleomycin model with reduced fibrosis and marker expression

Multicomponent Delivery Vehicle



Disease Background

Idiopathic pulmonary fibrosis (IPF) is a fatal lung disease marked by progressive, irreversible scarring. IPF involves chronic inflammation, fibroblast activation, and excess ECM deposition. Current therapies slow progression but do not reverse disease, highlighting the need for targeted treatments.

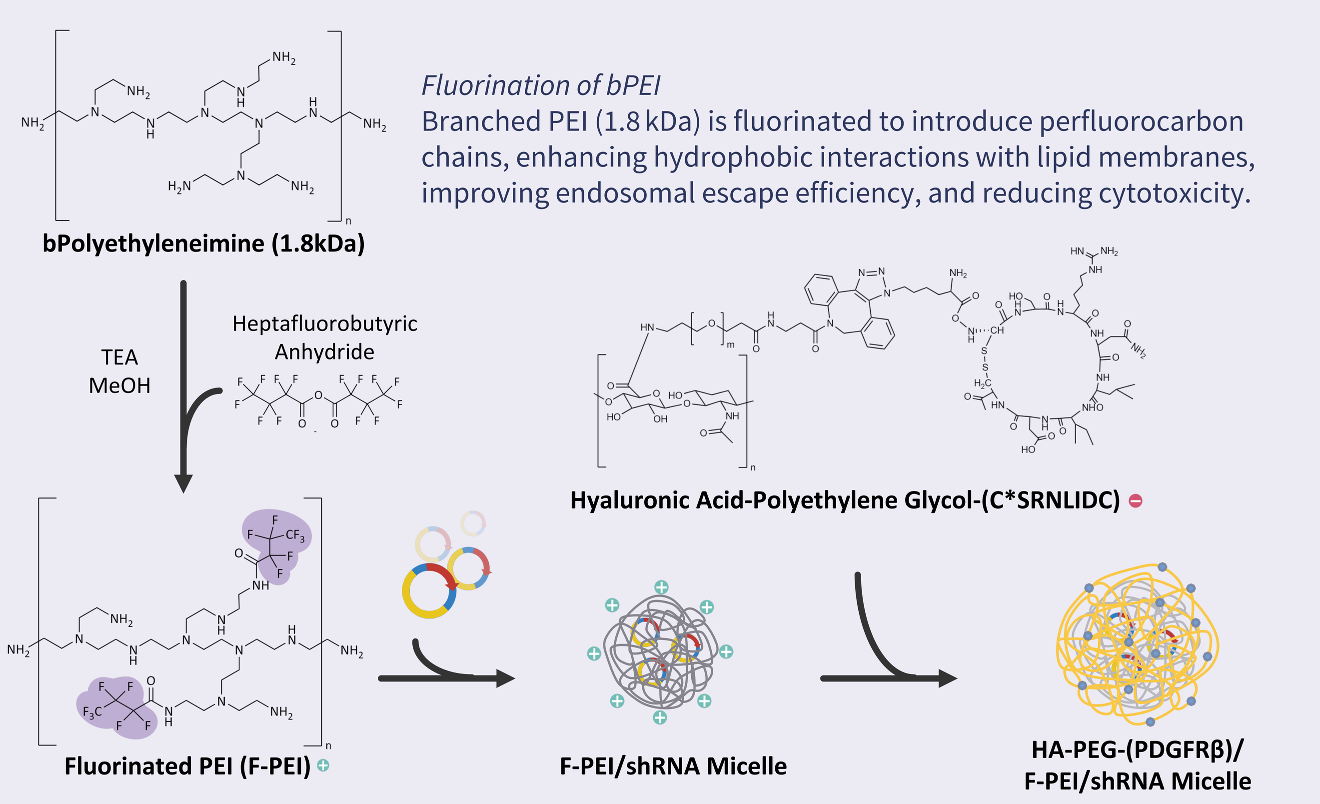


Healthy Alveola Intact epithelium and basement membrane support gas exchange and lung elasticity.

Injury & Inflammation Epithelial damage attracts immune cells that release cytokines driving fibrosis

Aberrant Wound Repair Injury activates fibroblasts, leading to ECM buildup and irreversible lung scarring

Synthesis and Assembly of shRNA/F-PEI–HA-PEG-(PDGFRβ) Core-Shell Micelle Nanostructures

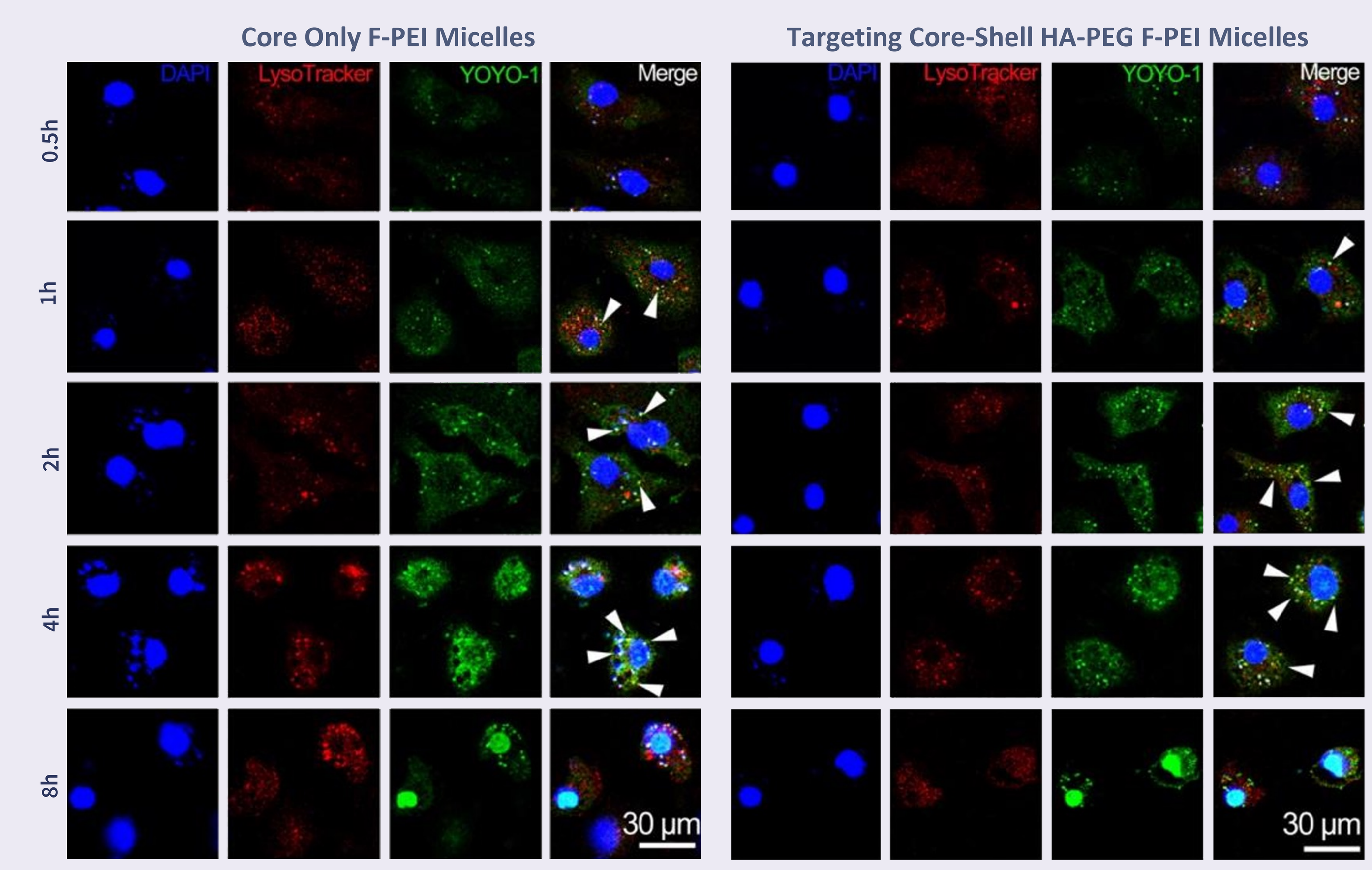


Fluorination of bPEI
Branched PEI (1.8 kDa) is fluorinated to introduce perfluorocarbon chains, enhancing hydrophobic interactions with lipid membranes, improving endosomal escape efficiency, and reducing cytotoxicity.

shRNA Complexation
Negatively charged shRNA rapidly self-assembles with cationic F-PEI via electrostatic interactions to form compact core micelles.

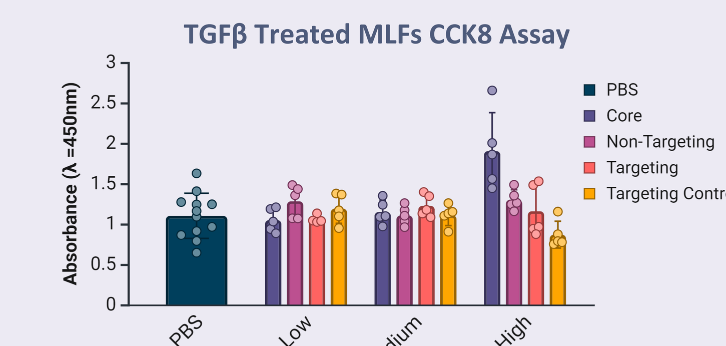
Targeted Core-Shell Nanostructure
A HA-PEG shell decorated with PDGFRβ-targeting peptide is grafted onto the F-PEI/shRNA core, imparting colloidal stability and receptor-mediated targeting

Core-Shell Structure Retains Effective Endosomal Escape

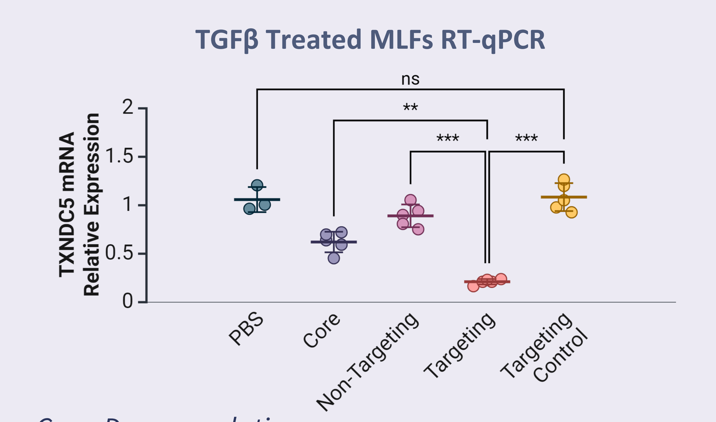


Endosomal Escape
Lysosomal trafficking assessed by colocalization of YOYO-1-labeled shRNA (green) with LysoTracker (red) indicates both core and core-shell particles achieve endosomal escape and nuclear accumulation. The HA-PEG shell slightly slows escape kinetics but does not abrogate delivery efficacy.

Cell Viability & TXNDC5 Knockdown

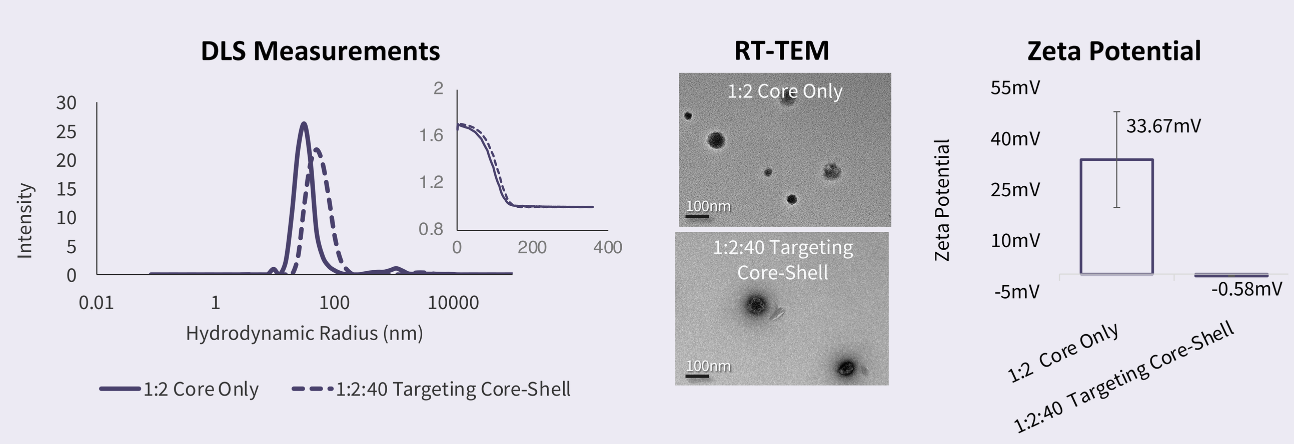


Cytotoxicity Assay
CCK-8 viability assay in murine lung fibroblasts was performed at increasing plasmid doses (0.5, 1, 2 µg/mL) and indicated retained viability in most groups.

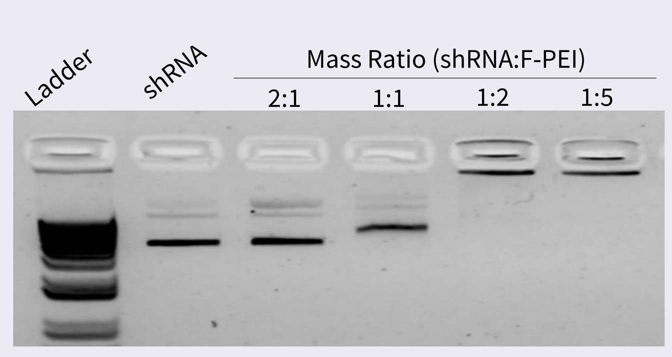


Gene Downregulation
RT-qPCR Analysis of TXNDC5 mRNA reveals significant knockdown in the targeted core-shell group.

Size & Morphology Characterization of Micelle Nanostructures

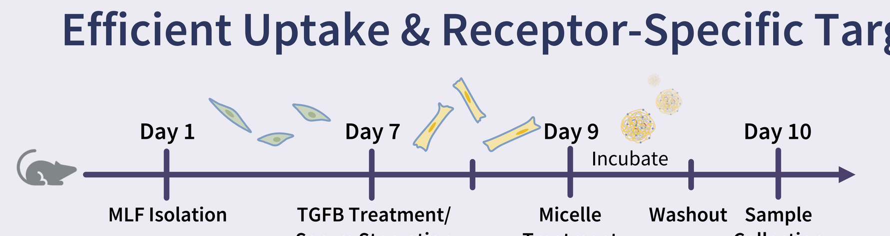


Size and Morphology Characterization
DLS reveals hydrodynamic radius of ~30 nm for cores and ~50 nm after shelling; TEM confirms uniform spherical morphology; zeta potential shifts from ~+34 mV (core) to ~-0.6 mV (core-shell).

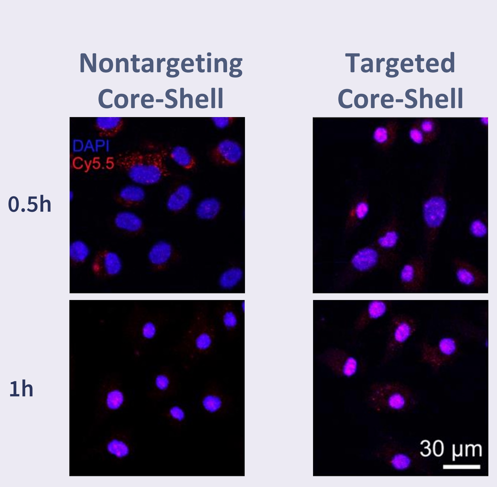


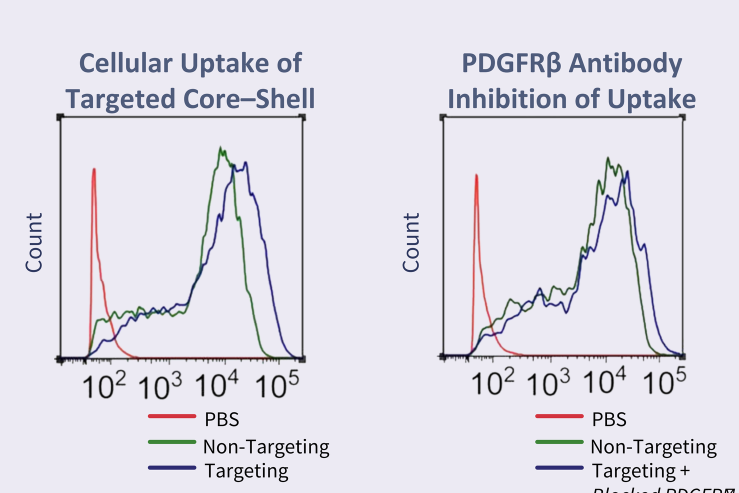
Retention Assay
Self-assembly with F-PEI enables efficient plasmid condensation. Gel electrophoresis confirms complete shRNA complexation at a 1:2 shRNA to F-PEI ratio.

Efficient Uptake & Receptor-Specific Targeting *In Vitro*



In Vitro Experiments
Murine derived lung fibroblasts (MLFs) are isolated & activated

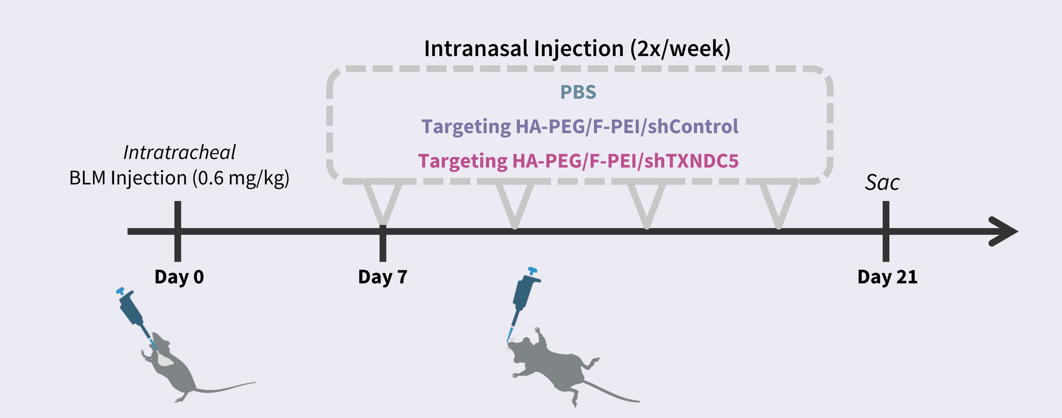




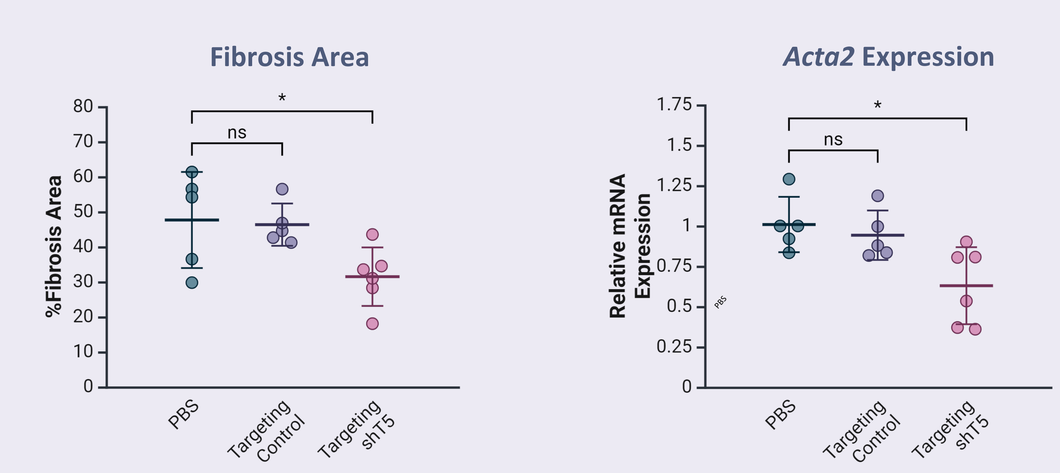
Cell Uptake Characterization
Confocal microscopy shows Cy5-labeled (red) micelles in fibroblasts at 0.5 and 1 h post-treatment. Nuclei stained DAPI (blue).

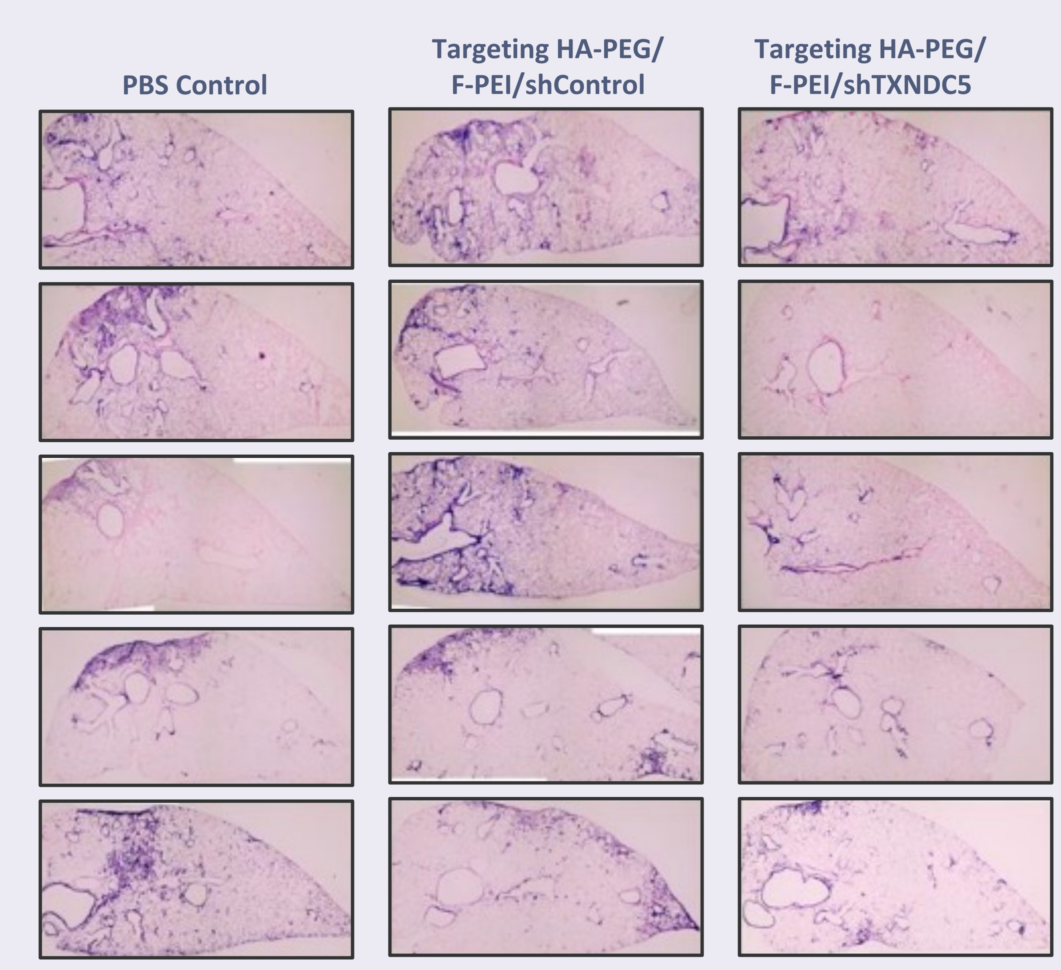
Receptor Specific Targeting
Flow cytometry shows higher uptake of the targeted formulation with inhibition after pre-saturation using free anti-PDGFRβ antibody.

In Vivo Studies Indicate Effective Reduced Fibrotic Burden & Downregulated Genetic Markers of Fibrosis

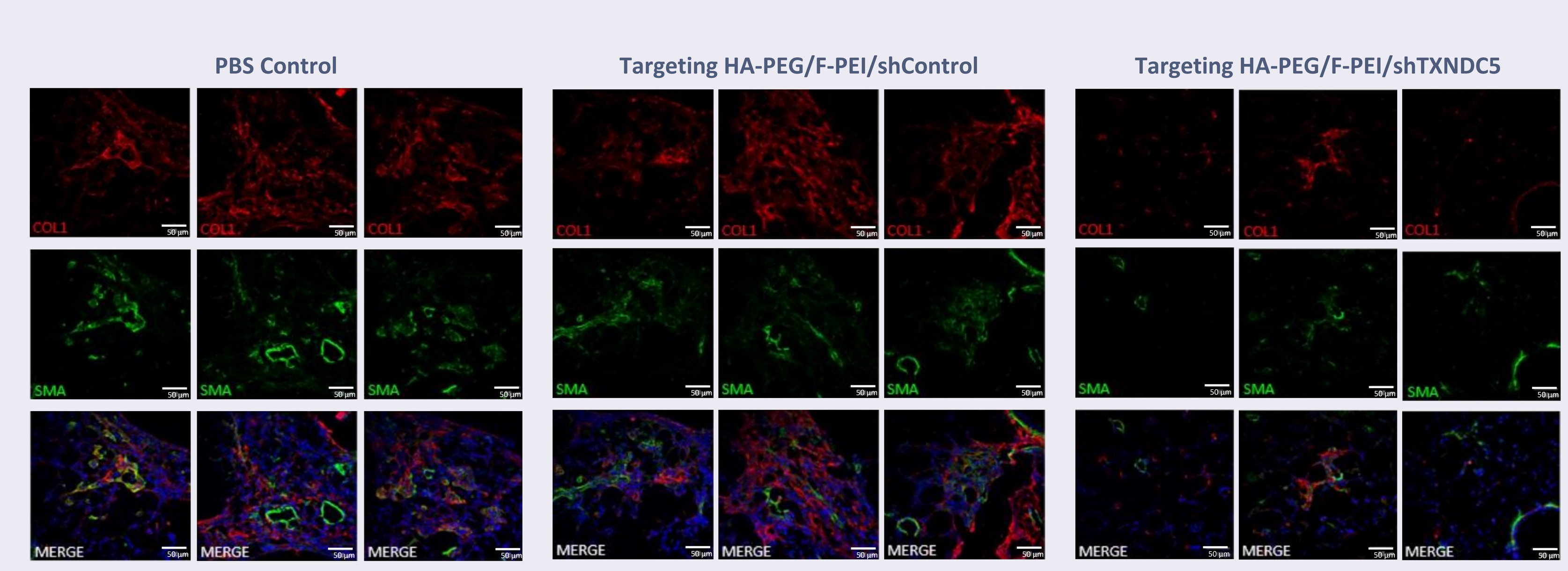


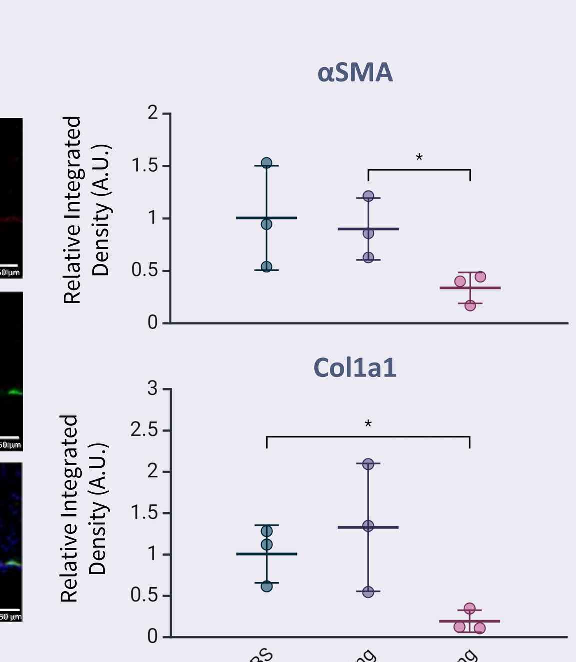
Bleomycin Murine Model of IPF
C57BL/6 mice receive intratracheal bleomycin on Day 0 to induce fibrosis. Micelles are delivered intranasally starting Day 7, twice weekly. Endpoint analyses at Day 21.





Lung Histology
Masson's Trichrome staining and relative Acta2 mRNA expression show high fibrosis in PBS and core- shell targeting control (carrying non-therapeutic plasmid) groups, indicating that the delivery vehicle alone does not alter fibrosis .TXNDC5 shRNA treatment reduces collagen deposition and Acta2 levels, confirming antifibrotic efficacy.





Fibrotic Marker Suppression
Immunofluorescence for α-SMA and COL1A1 shows strong fibrotic marker expression in PBS and targeting control groups. TXNDC5 shRNA treatment reduces α-SMA and COL1A1 staining, indicating effective in vivo suppression.

Future Perspectives

Future work will evaluate aerosolized delivery of the targeted core-shell micelles via nebulization, assessing particle stability, lung deposition, and biodistribution in healthy and fibrotic models. Additional studies will examine long-term lung function, immune response, and off-target effects to support safety and translation. The modular platform can be adapted for other RNA species, CRISPR systems, or small molecules, offering broad applicability for precision therapies.

References

Li, L., et al. (2017). *ACS Nano*, *11*(1), 95–111.
Lee, T., et al. (2020). *Nature Communications*, *11*(1), 4254.
Beljaars, L., et al. (2003). *Biochemical Pharmacology*, *66*(7), 1307–1317.
Lv, J., et al. (2014). *Journal of Materials Chemistry B*, *3*(4), 642–650.

Acknowledgments

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