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Session: Ocular Delivery

CRISPR-Cas Delivery Using Lipid-polymer Nanoplexes in Microneedle Patches for Corneal Dystrophy

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PHILADELPHIA, PA
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

Introduction

Corneal dystrophies are bilateral genetic disorders caused by mutations in specific genes, leading to progressive accumulation of irregular deposits in the cornea and vision loss. While genetic advances offer insights, targeted therapies remain limited, highlighting the potential of CRISPR/Cas technology.

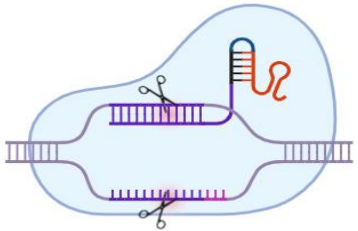
Normal eye

Corneal dystrophy eye

Mutated SLC4A11 gene

Congenital hereditary endothelial dystrophy (CHED)

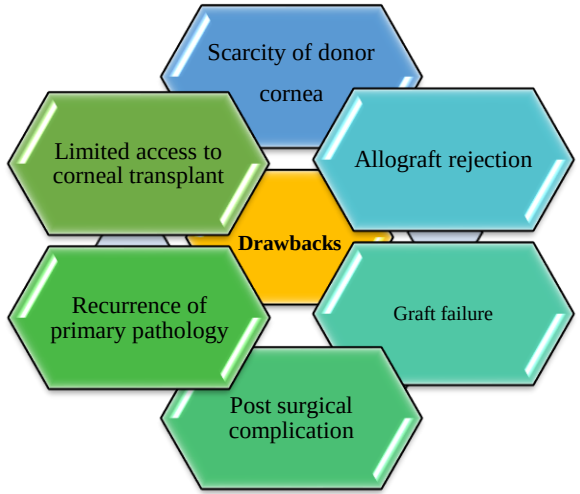
Gene Editing Tool



CRISPR/Cas System

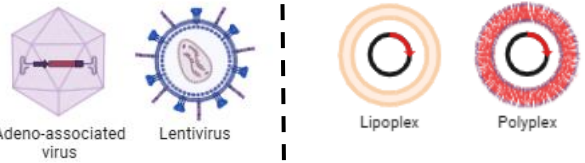
Current Treatment: surgical intervention

- ✓ Penetrating keratoplasty (PK),
- ✓ Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK),
- ✓ Descemet's Membrane Endothelial Keratoplasty (DMEK).



Delivery Strategies of CRISPR/Cas Components

Modes of delivery



Viral vectors

(Adeno-associated virus vectors)

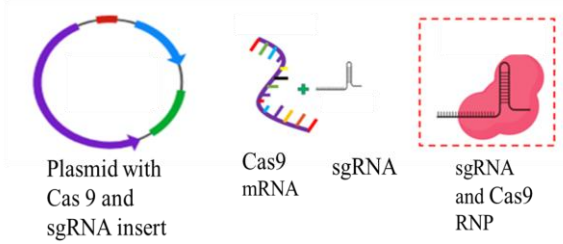
1. Limited packaging capacity (4.7 kb) for commonly used SpCas9 protein
2. Impose serious safety issues during therapeutic genome editing.

Non-viral vectors

(Cationic polymeric or lipidic nanocarriers)

1. Limited payload
2. Capacity
3. Cytotoxicity
4. Lack of tissue-specific targeting
5. Instability
6. Limited *in-vivo* application

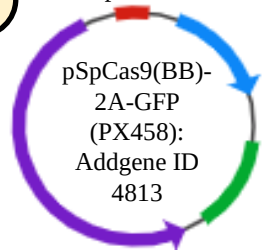
Delivery strategies



CRISPR/Cas Components

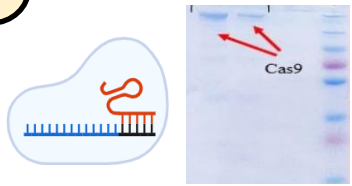
A

pDNA



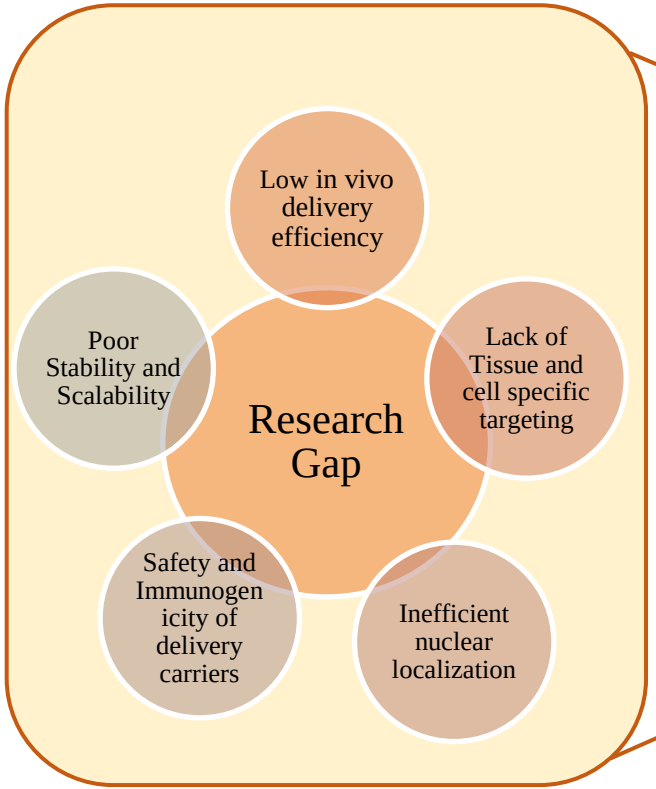
B

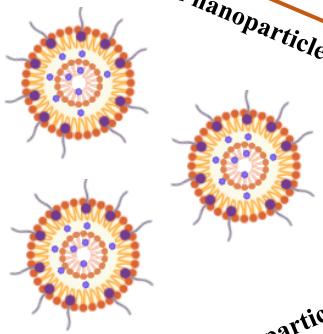
RNPs



Cas9 protein and SgRNA

Research Gaps and Objectives

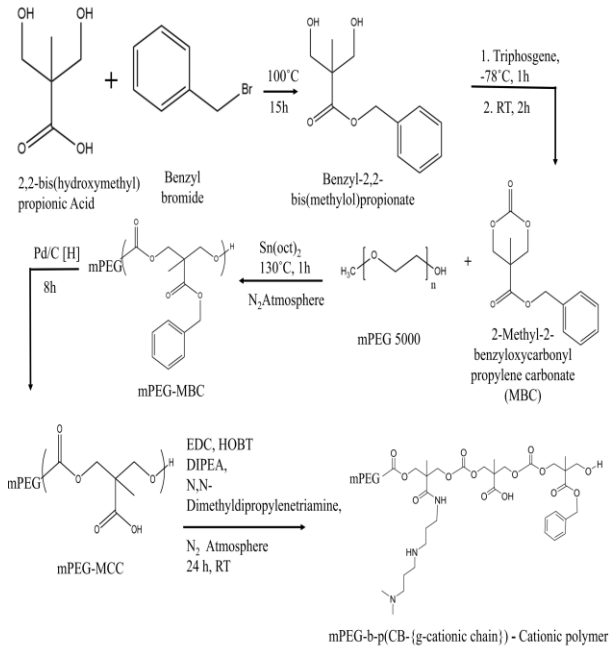




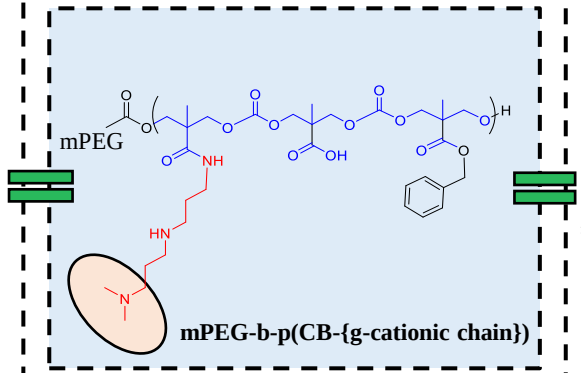
Objectives

1. Synthesis and characterization of cationic biodegradable polymer
2. Formulation and characterization of lipid-polymer hybrid nanoplexes
3. *In vitro* evaluation and cell culture studies of pDNA and RNP loaded nanoplexes
4. Lipid-polymer hybrid nanoplexes loaded into microneedle patch and its characterization

Synthesis Scheme of Biodegradable Cationic Polymer

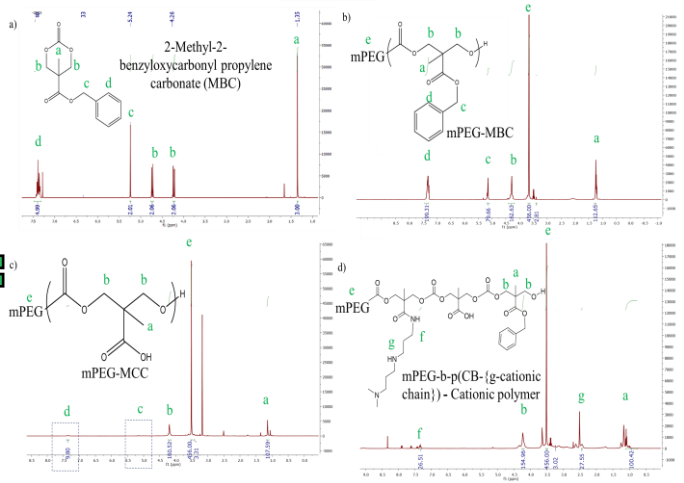


In-house Synthesized Biodegradable Cationic Polymer



Characterization of Biodegradable Cationic Polymer

1H NMR



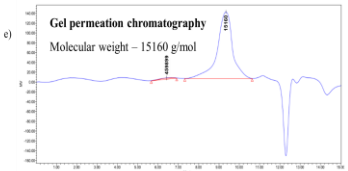


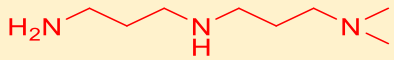
Table 1: Elemental analysis

Cationic polymer	Sample Weight (mg)	C%	H%	N%
mPEG-b-p(CB35-{g-Cation chain27})	10	48.49	5.57	6.90

Advantages

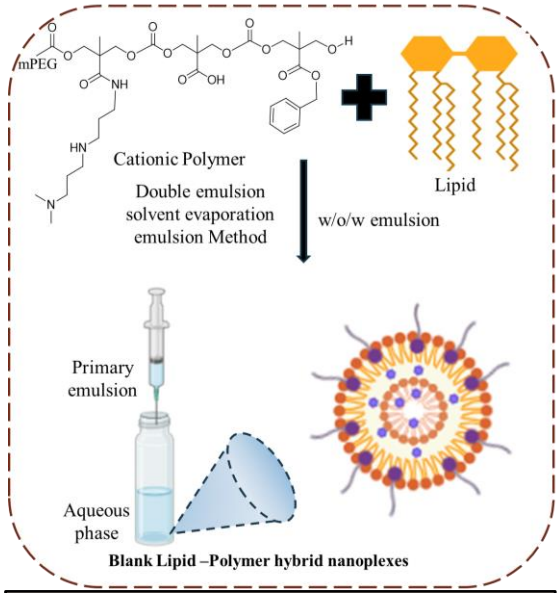
- Biodegradable
- Cationic charge
- Endo/lysosomal escape

Functional moieties: N,N-Dimethyldipropylenetriamine



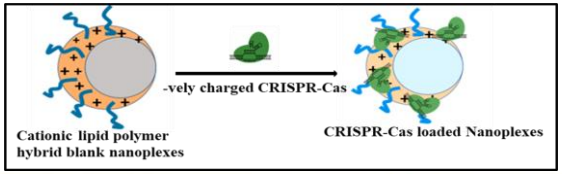
- ✓ Unit of Polymer Backbone: 35
- ✓ Unit of functional moieties: 27

Preparation of Blank Lipid-Polymer hybrid nanoplexes



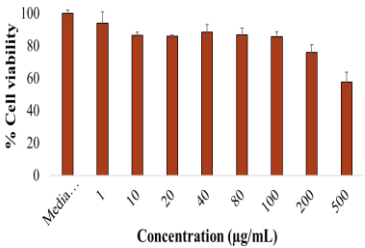
Blank Lipid –Polymer hybrid nanoplexes

Hypothesis based on electrostatic interaction

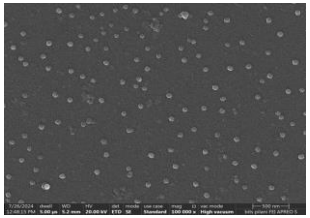


In vitro characterization of nanoplexes

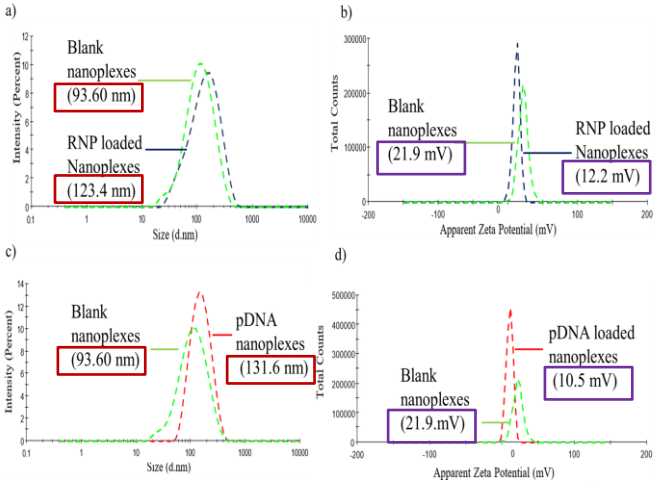
Cytotoxicity study 72 hr (SIRC cell line)



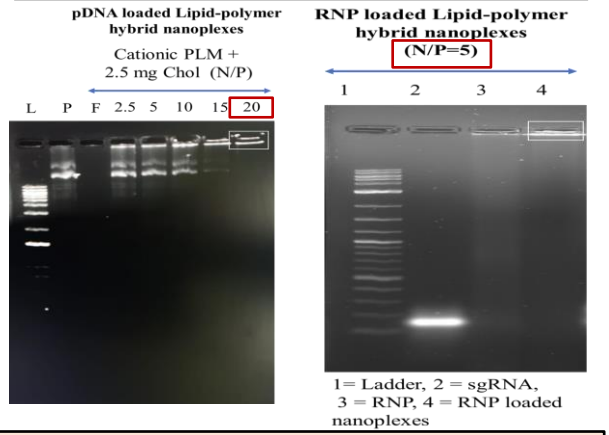
FESEM



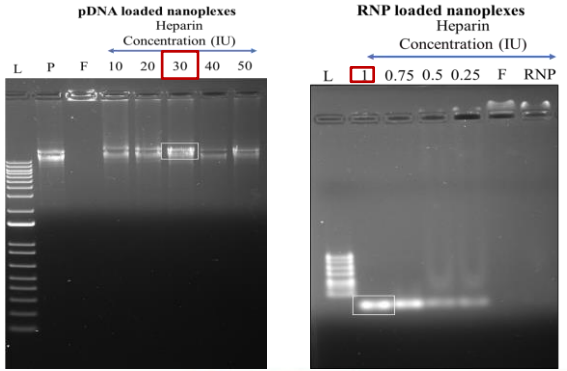
Particle size and zeta potential analysis



Complexation efficiency of nanoplexes

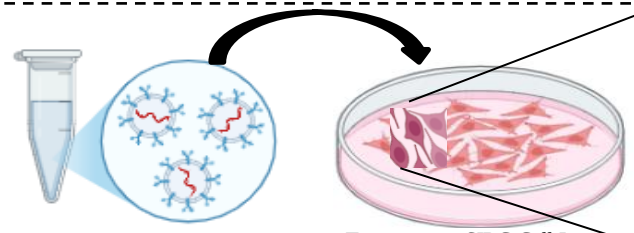


Heparin competition assay: Release of CRISPR-Cas component



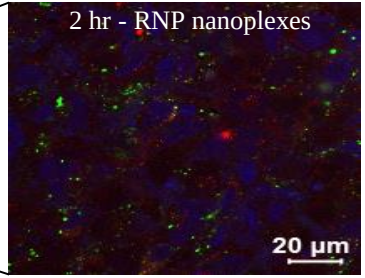
In-vitro RNPs transfection by lipid-polymer hybrid nanoplexes

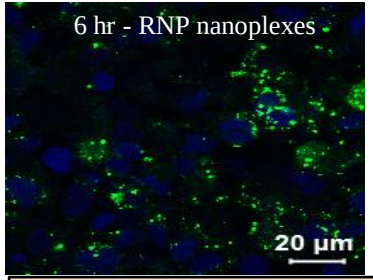
CRISPR/Cas loaded lipid-polymer hybrid nanoplexes



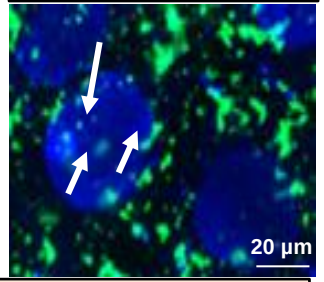
Treatment on SIRC Cell Line

Endo/lysosomal escape by lipid-polymer hybrid nanoplexes

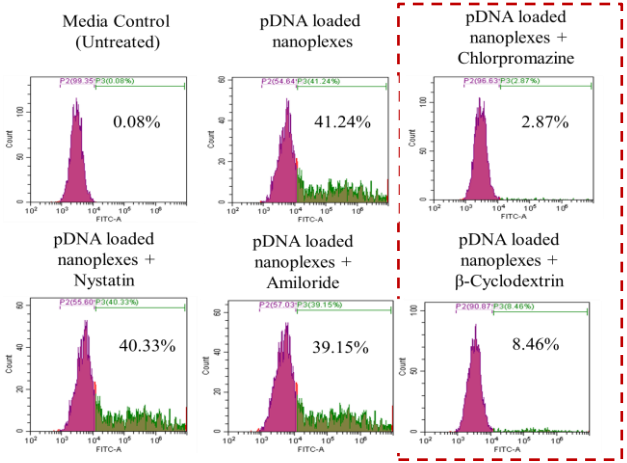




Nuclear Localization-24hr

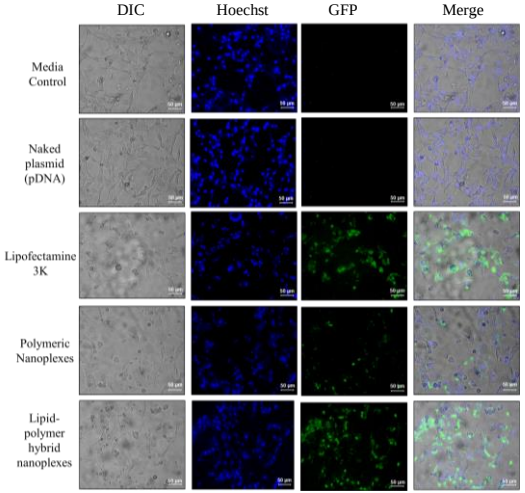


Endocytic uptake pathway study

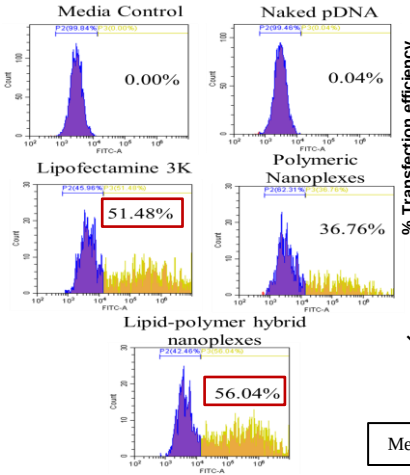


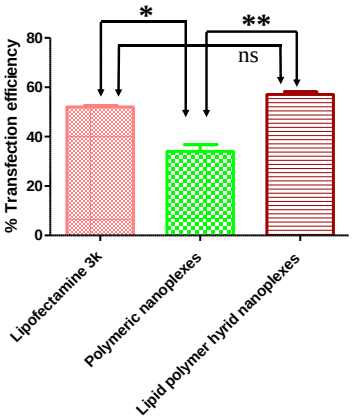
- ✓ Lipid raft mediated endocytic uptake pathway
- ✓ Clathrin mediated Pathway

In vitro qualitative transfection efficiency



In vitro quantitative transfection efficiency

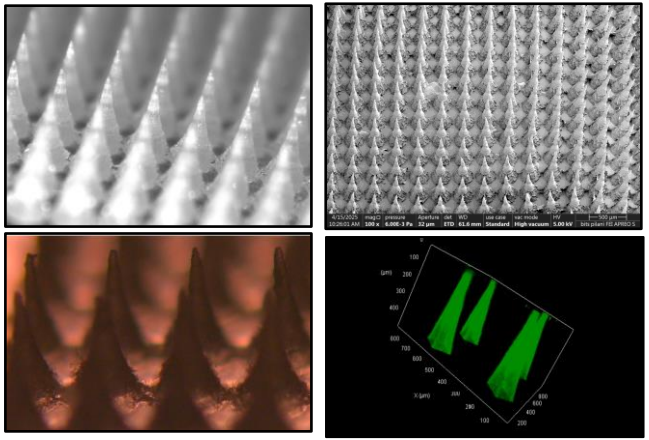




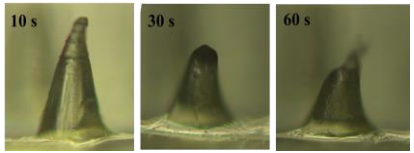
Mean±SD, SD=3, *p < 0.05; **p < 0.01

Characterization of microneedle patch

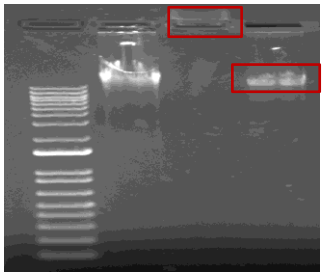
Morphological analysis of MN Patch



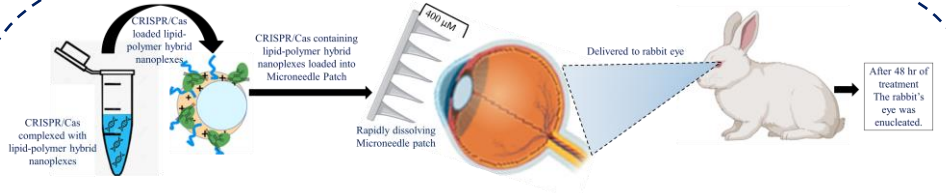
In vitro Dissolution of MN Patch and Integrity of Plasmid DNA



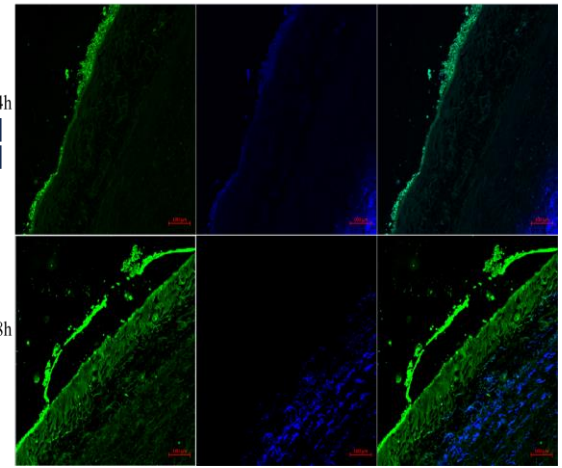
L: DNA Ladder; **P**: Plasmid DNA; **S1**: Plasmid DNA complexed with LPH formulation after 60 seconds of dissolution; **S2**: Naked plasmid DNA released from LPH after incubation with heparin.

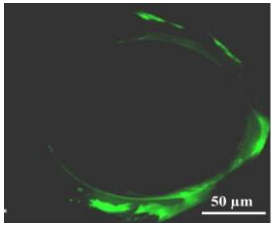


In vivo ocular distribution and histopathological analysis

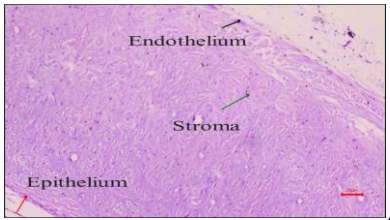


GFP in Rabbit Cornea: 48hr of post treatment





H&E analysis:Non-Toxic

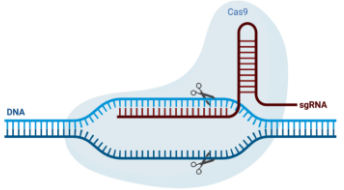


Conclusion

1. Developed a CRISPR-Cas delivery system using lipid-polymer hybrid nanoplexes for targeted gene editing in corneal cells.
2. Achieved high transfection efficiency (~60%) with minimal cytotoxicity in HEK293 and SIRC cells.
3. Microneedle patches enabled CRISPR-Cas delivery with no toxicity in corneal layers which was confirmed by Ex vivo (Porcine) and in vivo (rabbit) studies.
4. This work presents a CRISPR-Cas delivery platform utilizing electrostatic interactions for efficient delivery and distribution in corneal cells

Indian Patent: Chitkara Deepak, **Guha Sonia** Lipid-Polymer Hybrid Nanoplexs Delivery System and Method of Synthesis. Filed an Indian patent **202511014451** on19th February 2025.

Future Perspective



To check **gene editing efficiency** in mutated gene in cornea, further evaluation in

- ✓ Patient-derived human corneal endothelial cells
- ✓ Disease animal of C57BL/B6 mice/ rabbit cornea

Major References:

1. Zhang et al, Macromolecular rapid communications, 2019
2. Sahel et al, JMCB, 2022
3. Wan et al, Science advances, 2021
4. Sahel et al, Macromolecular Rapid Communications, 2023

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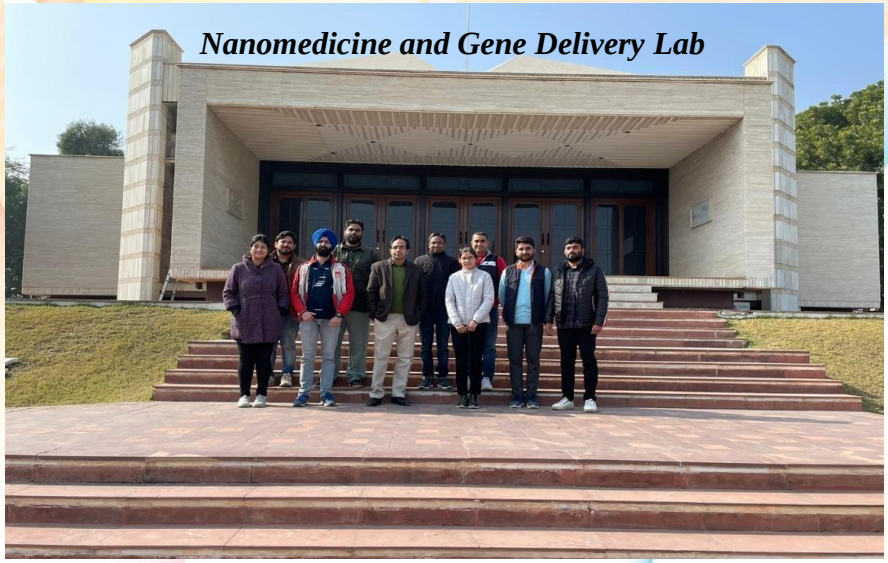
Department of Science and Technology, Government of India (Project no. CRG/2022/004265)

Thank You



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1. ***BITS Pilani, Pilani Campus, RJ, India***
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3. अनुसंधान नेशनल रिसर्च फाउंडेशन
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