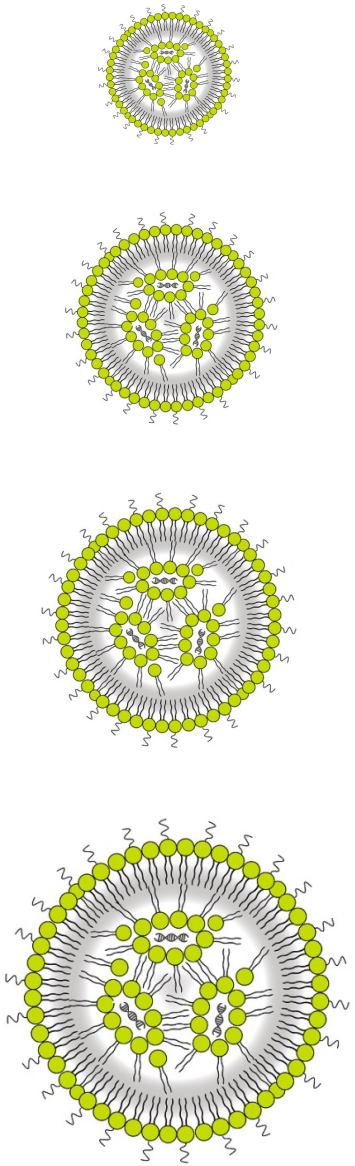
Presentation and Discussion of the Research Paper

The Effects of PEGylation on LNP Based mRNA Delivery To The Eye

By: Anfal Ibrahem

Research Topic & Seminar (501)

Tue 15th of June 2021



Outline

I. Introduction

A. PEGylation

B. Lipid Nanoparticles

II. Material and Method

A. Animals Selection

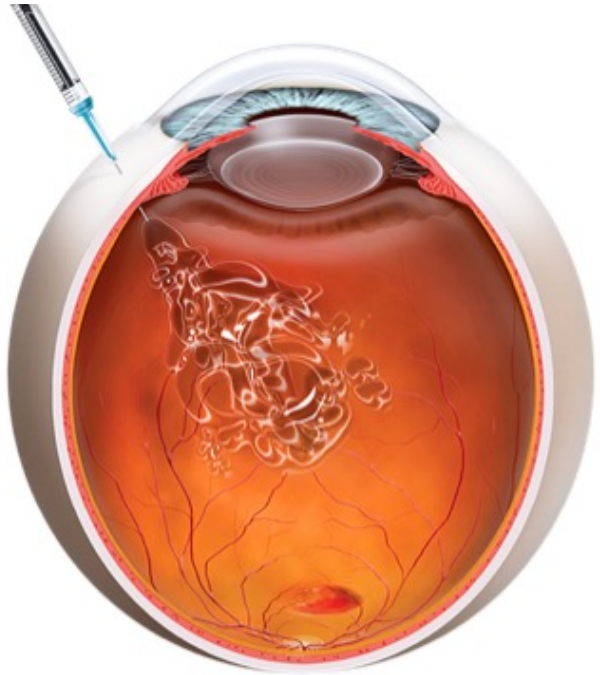
B. Nanoparticle Formulation and Characterization

C. Injections

D. In-vivo Bioluminescent Imaging

E. Fundus Photography

F. Immunohistochemistry



III. Result and Discussion

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B. The Effects of LNP size and DSPC Modification Post-subretinal Injection

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PLOS ONE

Research Paper

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The Foundation for The Gator Nation

The effects of PEGylation on LNP based mRNA delivery to the eye

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Abstract

Gene therapy is now an effective approach to treat many forms of retinal degeneration. Delivery agents that are cell-specific, allow for multiple dosing regimens, and have low immunogenicity are needed to expand the utility of gene therapy for the retina. We generated eight novel lipid nanoparticles (LNPs) ranging in size from 50 nm to 150 nm by changing the PEG content from 5% to 0.5%, respectively. Subretinal injections of LNP-mRNA encoding luciferase revealed that 0.5% PEG content within nanoparticles elicits the highest expression. Similar injections of LNP delivered cre mRNA into Ai9 mice revealed cell-specific protein expression in the retinal pigment epithelium (RPE), confirmed by fundus photography and immunohistochemistry of whole globe cross-sections. To investigate mechanisms of LNP delivery to the eye, we injected mCherry mRNA using the subretinal approach in *apoE*$^{-/-}$ and *Mertk*$^{-/-}$ mice. RPE transfection was observed in both mouse models suggesting that LNP intracellular delivery is not solely dependent on apolipoprotein adsorption or phagocytosis. To investigate LNP penetration, particles were delivered to the vitreous chamber via an intravitreal injection. The 0.5% PEG particles mediated the highest luciferase activity and expression was observed in the Müller glia, the optic nerve head and the trabecular meshwork, but failed to reach the RPE. Overall, particles containing less PEG (~150 nm in size) mediated the highest expression in the eye. Thus far, these particles successfully transfect RPE, Müller cells, the optic nerve head and the trabecular meshwork based on route of administration which can expand the utility of LNP-mediated gene therapies for the eye.

Introduction

What do you know about blindness?

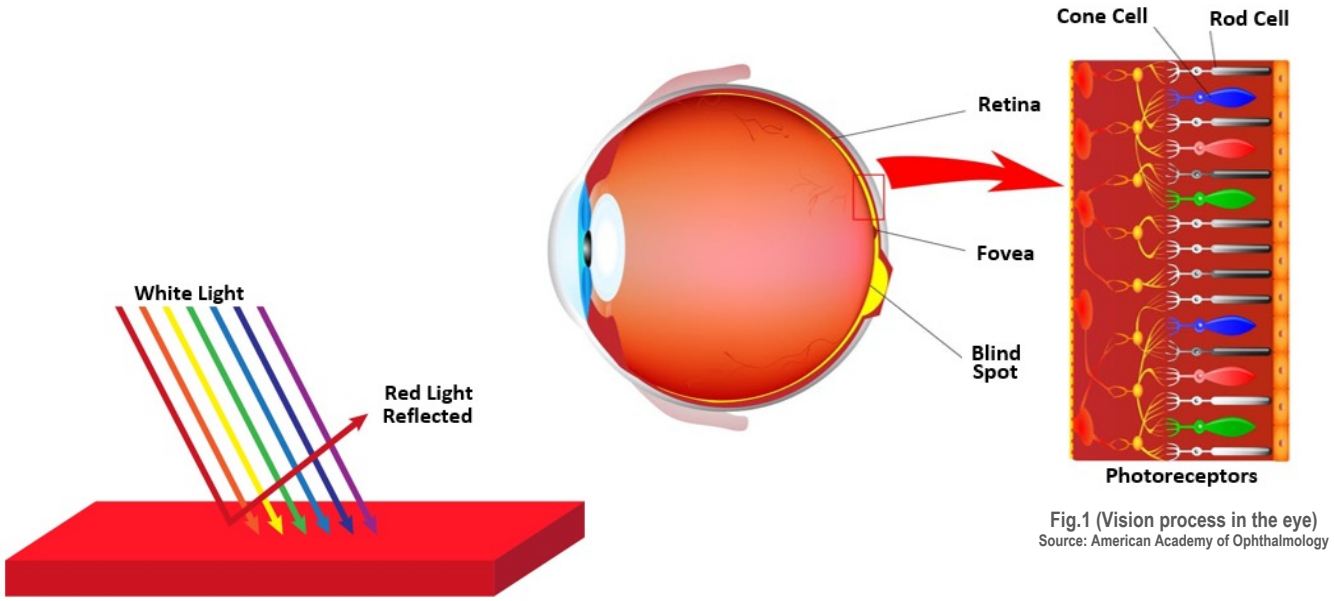


Fig.1 (Vision process in the eye)
Source: American Academy of Ophthalmology

Retinal Blindness
(Mutation RPE65)

How you would treat it?

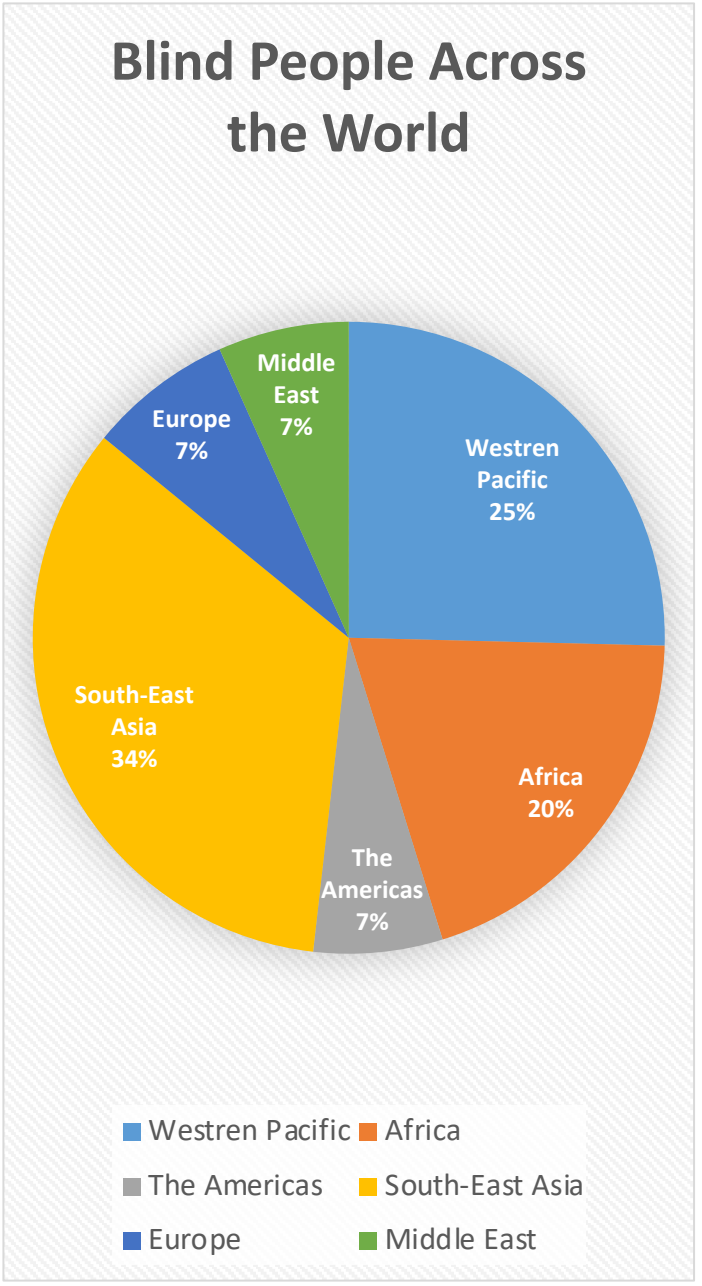


Fig.2 (Blindness around the world)
Source: International Center for eye Health

Gene Therapy

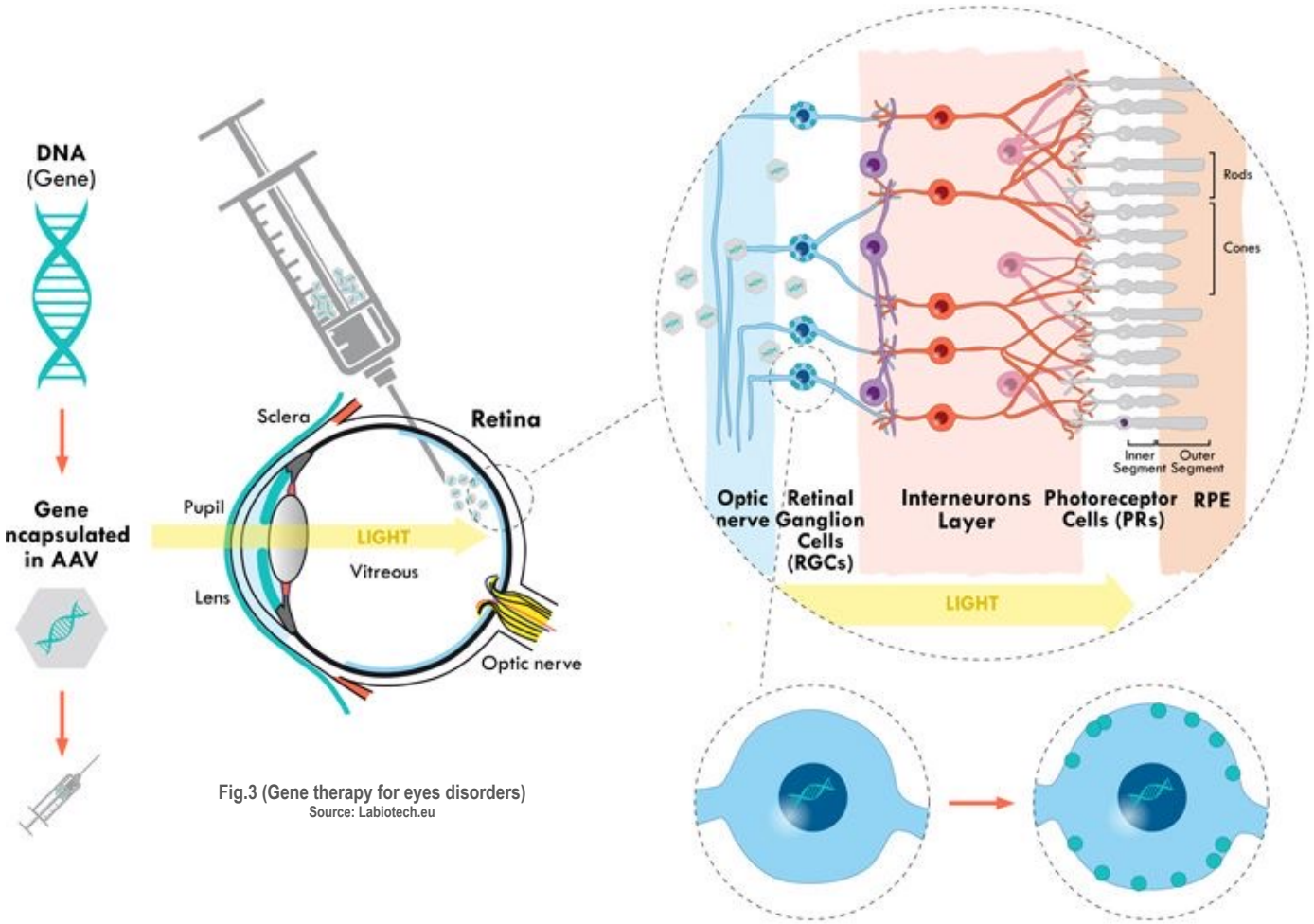
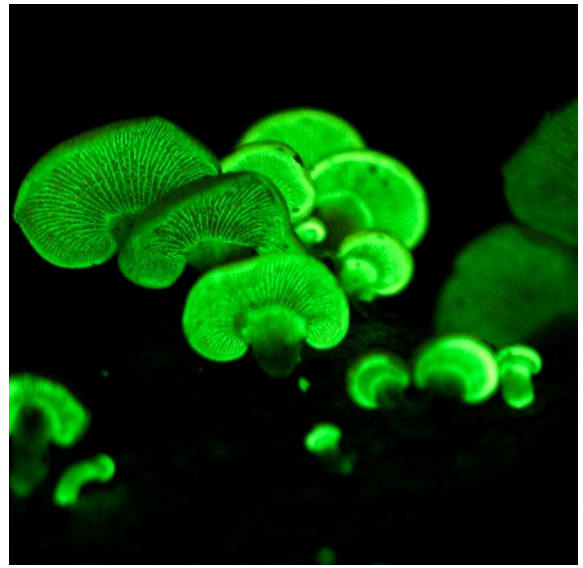
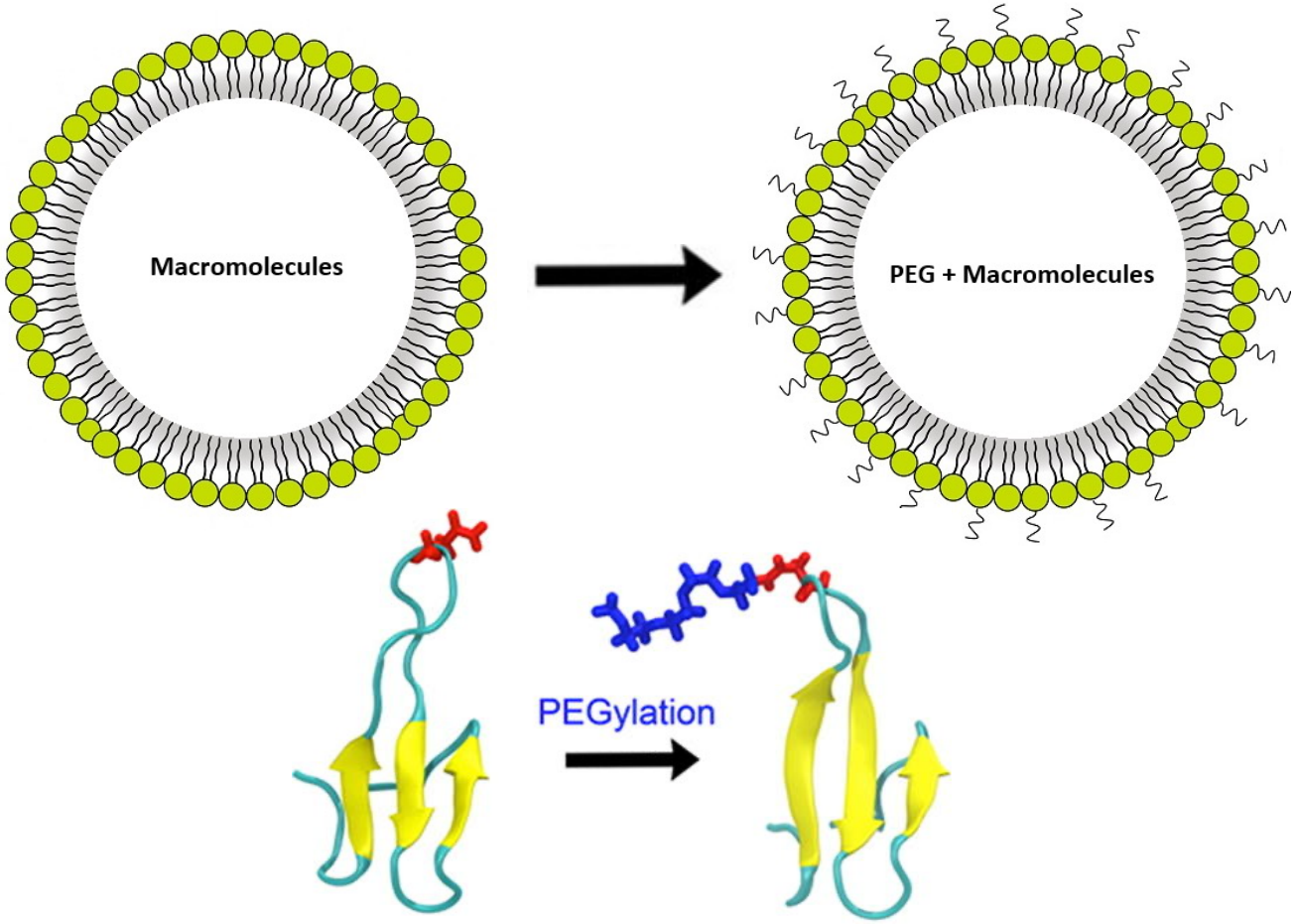


Fig.3 (Gene therapy for eyes disorders)
Source: Labiotech.eu



Luciferin → Bioluminescent

PEGylation



What is PEGylation?

How it works?

PEGylation is the covalent coupling of Non-Toxic Hydrophilic Poly ethylene glycol (PEG)

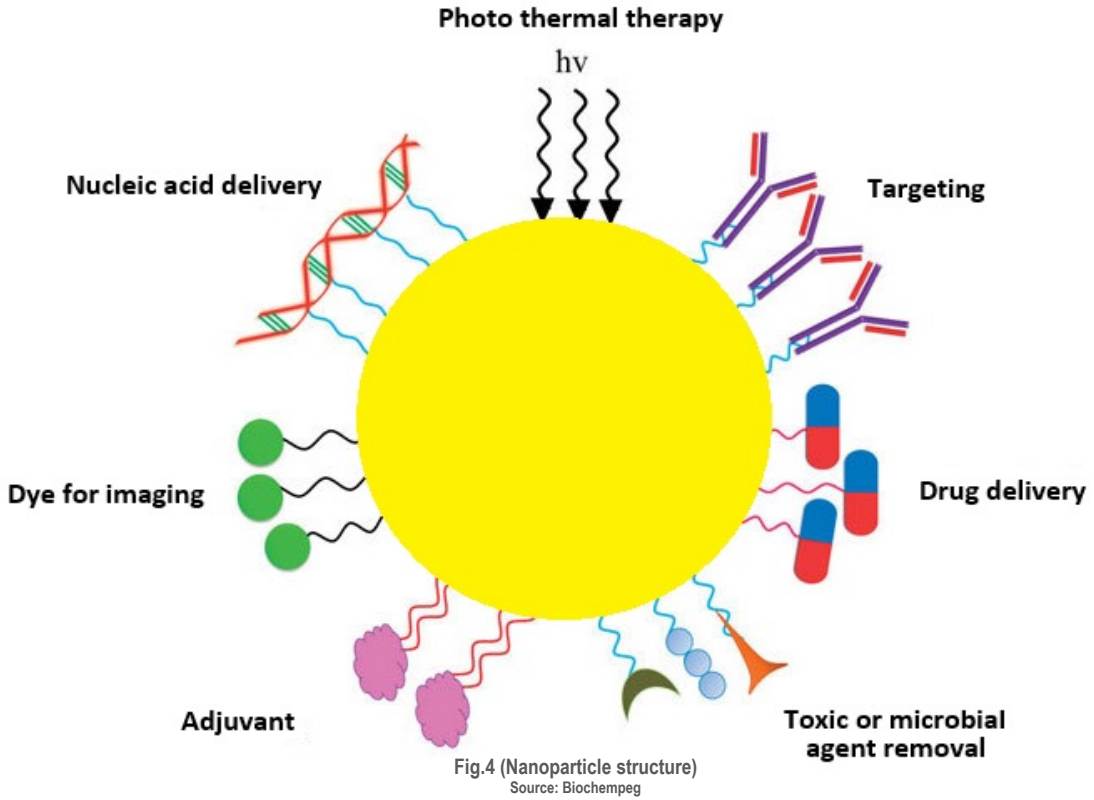


Fig.4 (Nanoparticle structure)
Source: Biochempeg

Lipid Nanoparticles

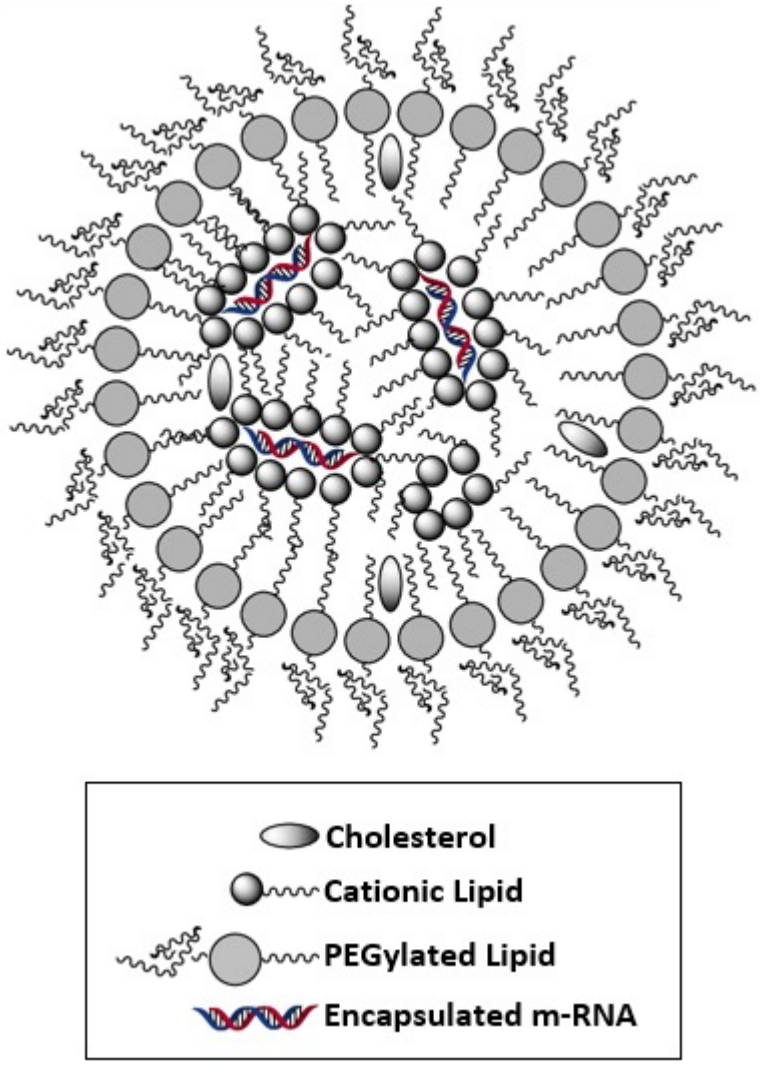


Fig.5 (Lipid Nanoparticles structure)
Source: ResearchGate

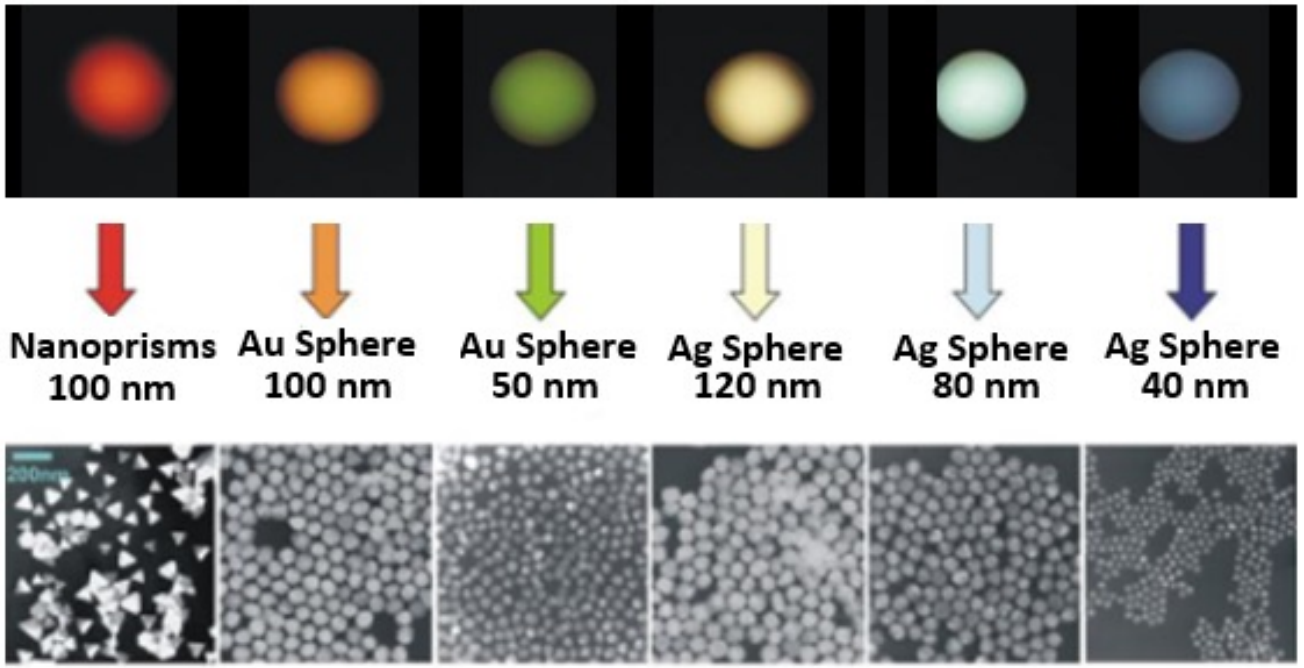


Fig.6 (Lipid Nanoparticles size)
Source: ResearchGate

What is the role of Lipid Nanoparticles?

Gene Delivery Vehicle

Animals Selection

BALB/c Mice

↓

Produce plasmacytomas (monoclonal antibody) following injection

Fig.7(Mice selection)
Source: The Jackson Laboratory

apoE Mice

↓

Homozygous for Apoe^{tmUnc} mutation which show increase in total plasma cholesterol

C57BL6 Mice

↓

General purpose strain carrying both spontaneous and induced mutations

Mertk Mice

↓

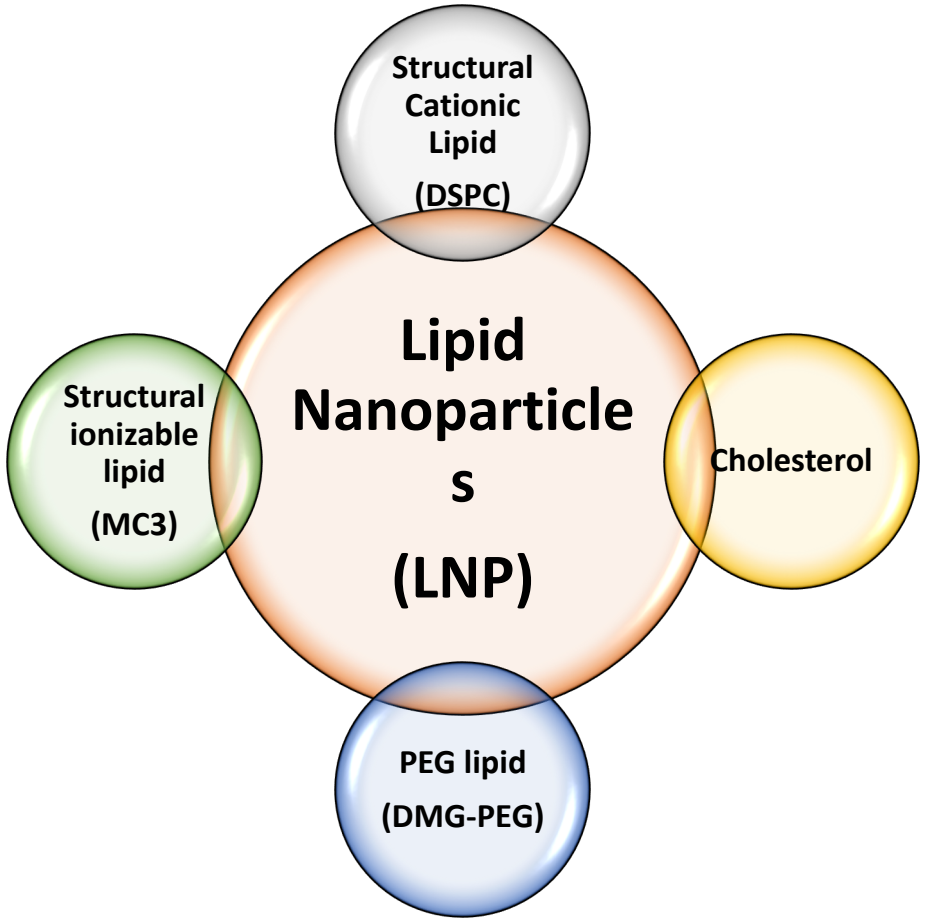
Exhibit an increase in spleen size and decrease in monocyte response and complete photoreceptor degradation

Ai9 Mice

↓

Prevent transcription of red fluorescent protein variant tdTomato

Nanoparticle Formulation and Characterization



How LNPs functioned?

LNPs Composition	Description
MC3	Unitized cationic lipids that is used for making LNPs.
DSPC	Type of phospholipid that is used for making LNPs.
DMG-PEG2k	Used with MC3 to form cationic nanoparticles that can encapsulate mRNA
L-7202	mRNA express Luciferase protein
L-7211	mRNA encoding Cre recombinase
L-7203	mRNA encoding fluorescent protein mCherry

Table.1 (Lipid Nanoparticles composition)
Source: Anfal's

LNPs were formulated

Microfluidic Mixing

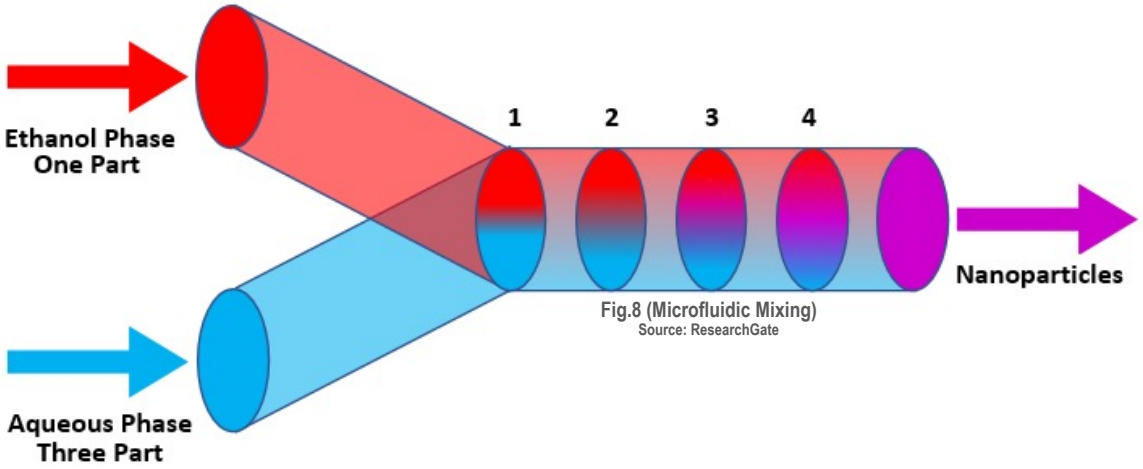


Fig.8 (Microfluidic Mixing)
Source: ResearchGate

Ethanol Phase	Aqueous Phase
MC3	mRNA + 50mM Citrate Buffer pH4
DSPC	
DMG-PEG2K	
Cholesterol	

The percentage of DMG-PEG was changed

0.5% 1.5% 3% 5%

Why?

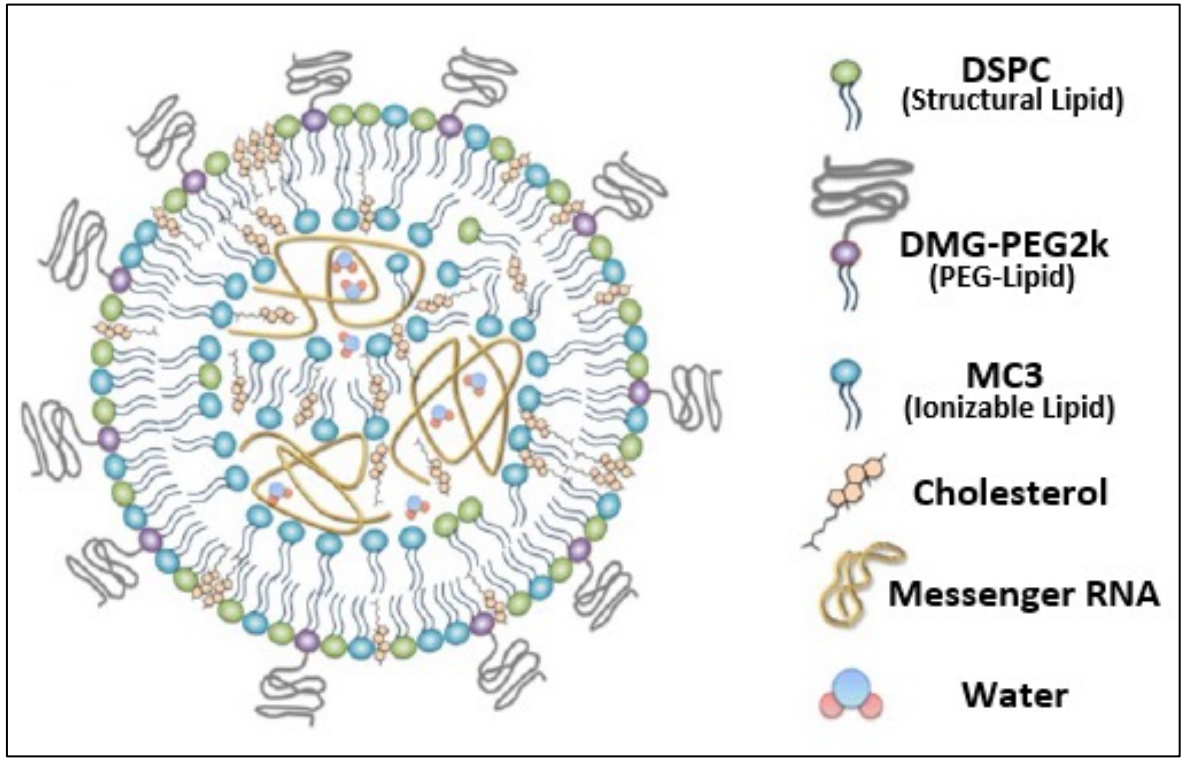
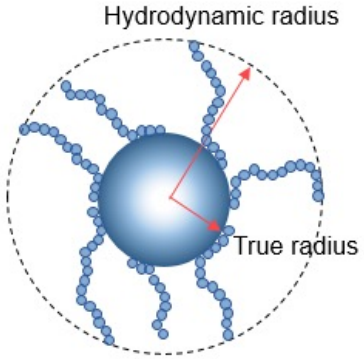


Fig.9 (Lipid Nanoparticles characterization)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

The LNPs solution was subjected to buffer exchange with PBS pH 7.2

LNPs Characterization

Hydrodynamic radius



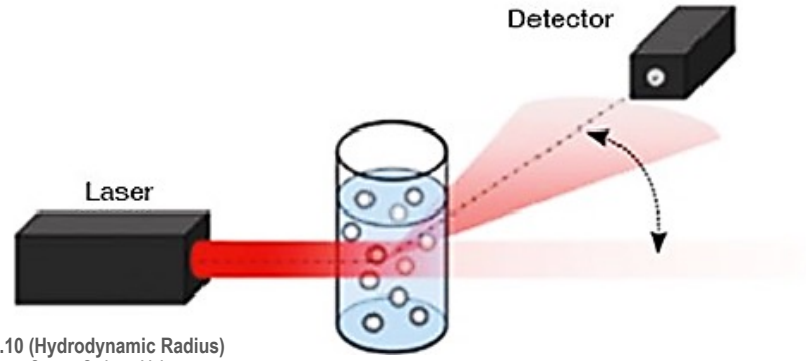


Fig.10 (Hydrodynamic Radius)
Source: Springer Link

Dynamic Light scattering

The generated particles size range from 150nm to 50 nm

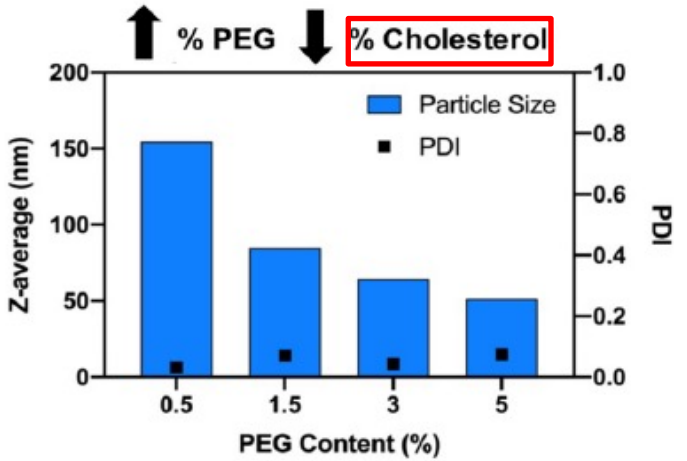


Fig.11 (Bar graph showing the size and PDI of one set of particles when percentage of PEG is increased and modulated against cholesterol)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

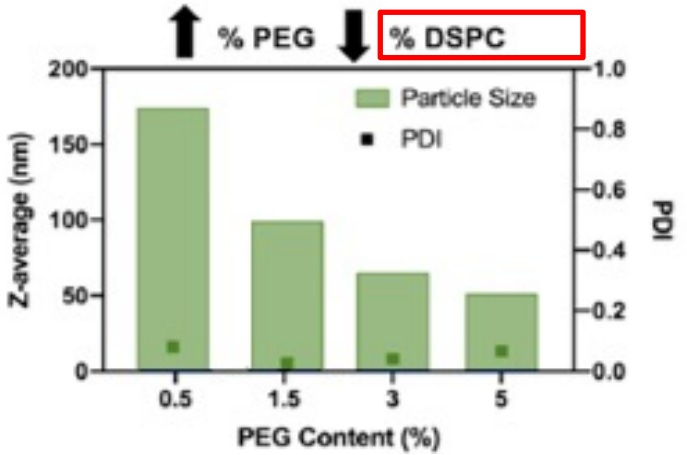


Fig.12 (Bar graph showing the size and PDI of one set of particles when percentage of PEG is increased and modulated against DSPC)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

mRNA encapsulation efficiency was measured

↓

Quantification RNA in solution

LNP #	DMG-PEG (%)	Cholesterol (%)	DSPC (%)	MC3 (%)	Encapsulation Efficiency (%)
1	0.5	39.5	10	50	97.8
2	1.5	38.5	10	50	96.6
3	3	37	10	50	98.4
4	5	35	10	50	94.8
5	0.5	38.5	11	50	96.2
6	1.5	38.5	10	50	95.8
7	3	38.5	8.5	50	97.2
8	5	38.5	6.5	50	97.0

Table. 2 (LNP components and characterization)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

How PEGylation and size may impact intracellular delivery?

Injection

Two Type of Injection

Subretinal

Intravitreal

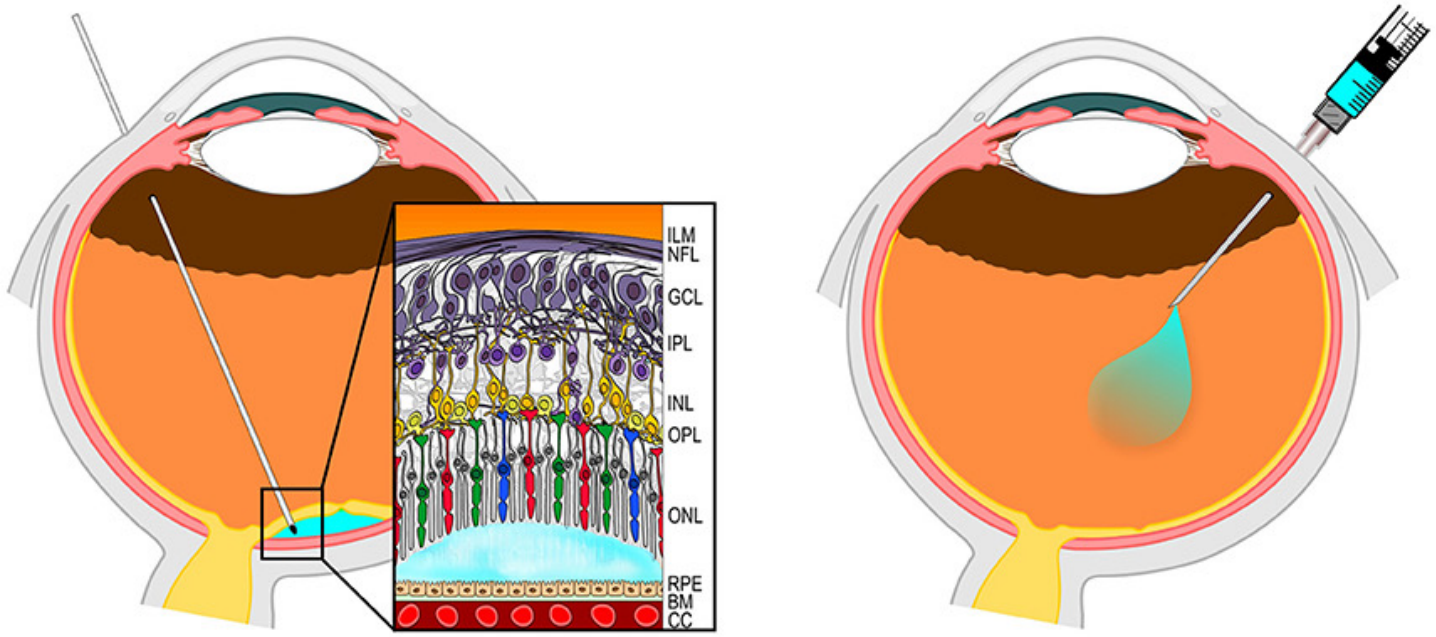


Fig.13 (Type of injection subretinal versus intravitreal)
Source: Mini Review Retinal Gene Therapy

Prior to injection

Mice were administered with

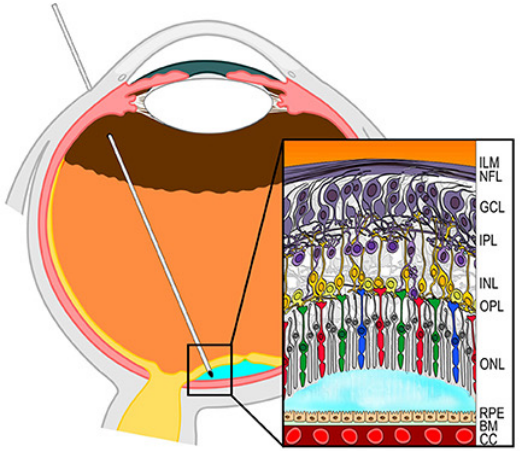
0.5% proparacaine
Eye drop numb

1% tropicamide
Widen the pupil

2.5% phenylephrine
Relieve sense congestion and pressure

Ketamine
anesthetic

subretinal



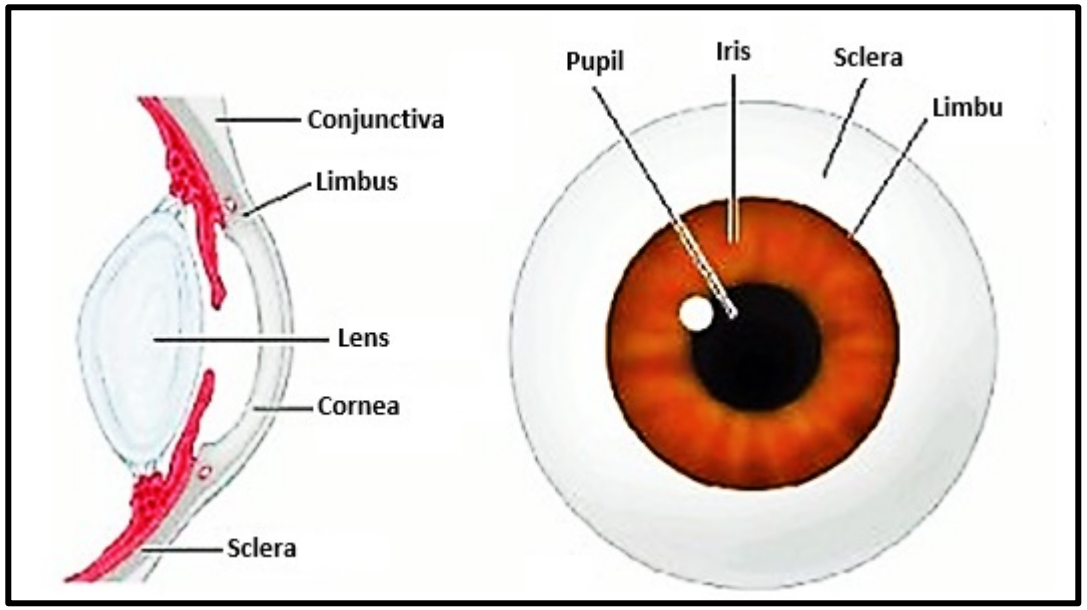


Fig.14 (Eyes structure)
Source: Louisville Eye Center

2.5% hypromellose
was placed over the eyes to relive the dryness and irritation

↓

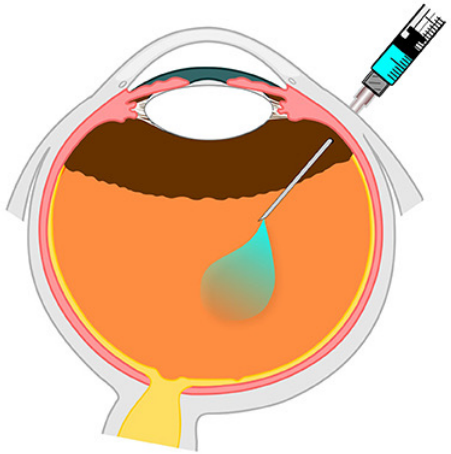
Going through the scleral to the limbus using a Hamilton syringe

↓

2% fluorescent solution was added to the PBS or LNPs (Luciferase – Cre – mCherry)

Was delivered to Retina

Intravitreal



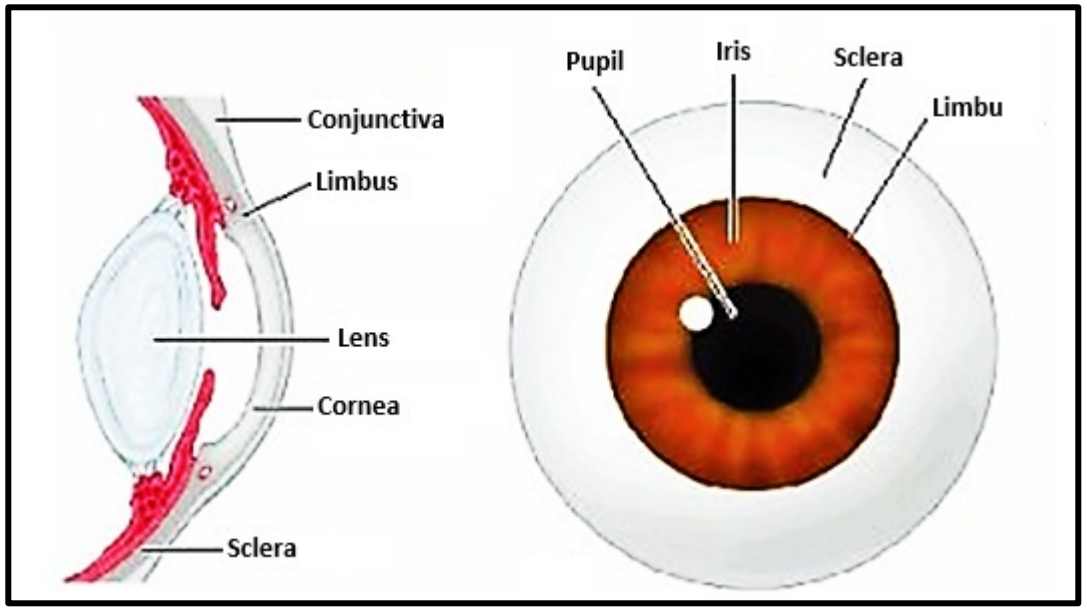


Fig.14 (Eyes structure)
Source: Louisville Eye Center

2.5% hypromellose
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↓

Going through the scleral to the limbus using a Hamilton syringe

↓

2% fluorescent solution was added to the PBS or LNPs (Luciferase – Cre – mCherry)

Was delivered to virtuous chamber

In-vitro Bioluminescent Imaging

Mice were injected with 150mg of Luciferin

↓

Bioluminescent imaging was conducted on the IVIS Spectrum

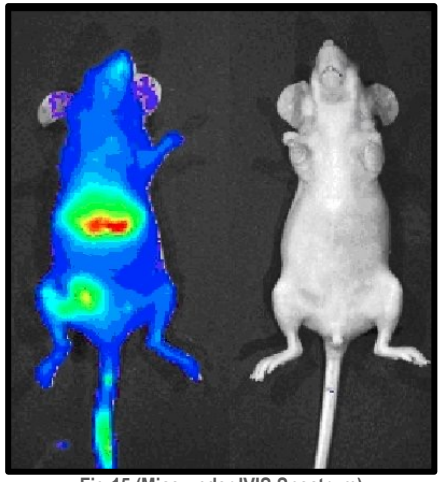


Fig.15 (Mice under IVIS Spectrum)
Source: ResearchGate

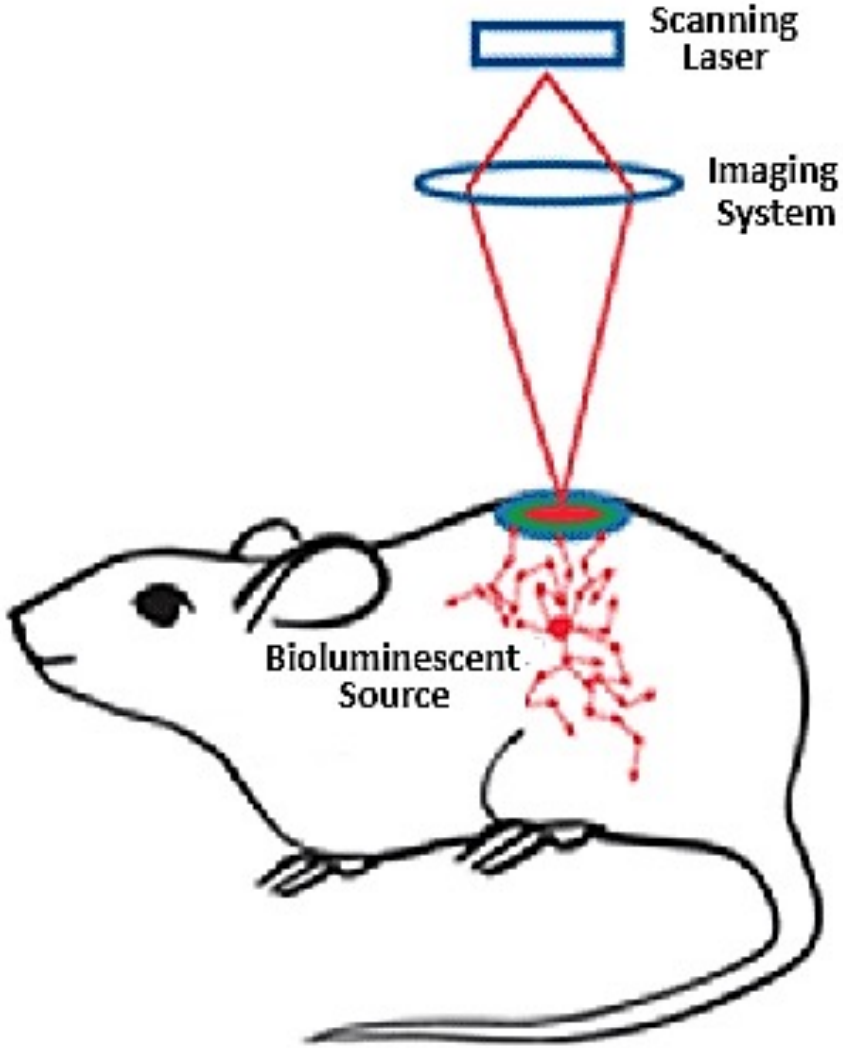


Fig.16 (Mice under IVIS Spectrum)
Source: ResearchGate

Analysis image for specific region

Fundus Photography

Live retinal imaging was performed with Micron IV

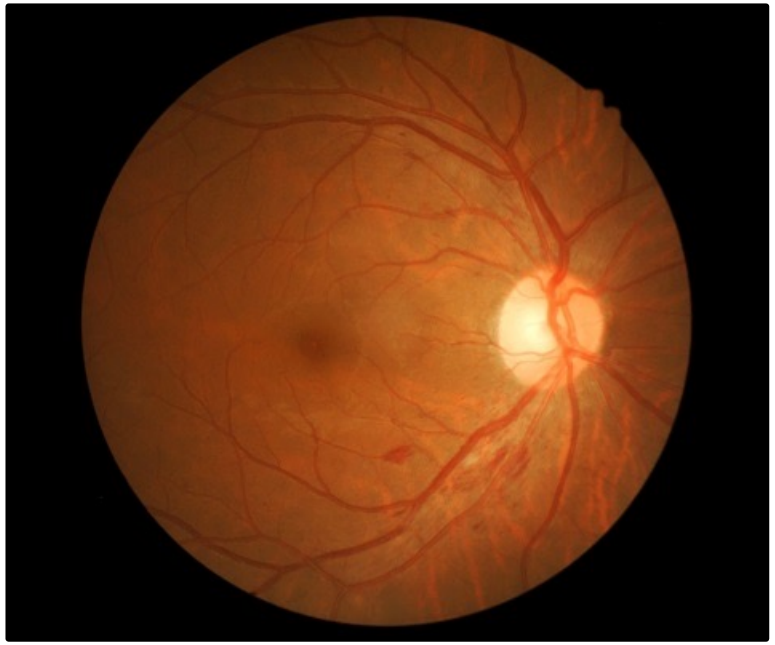


Fig.17 (Fundus Photography)
Source: New Jersey Vision Associate

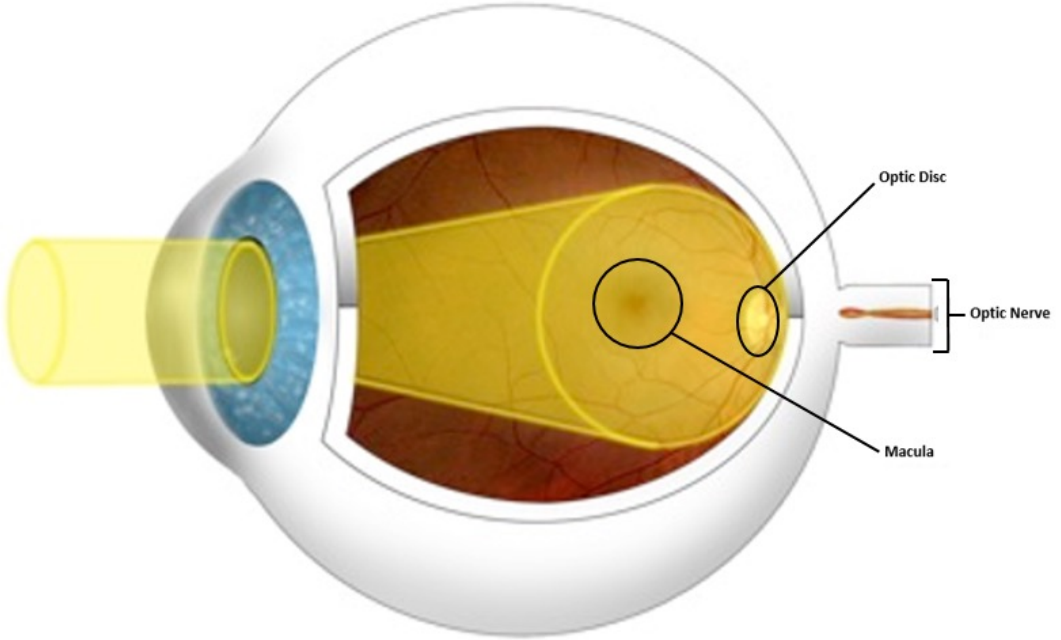


Fig.18 (Micron Iv image)
Source: The University of British Columbia

General retinal health

To capture td Tomato fluorescent protein

Immunohistochemistry

Mouse eyes were enucleated

↓

Fixation
49% paraformaldehyde

Sectioning
microtome

Staining
10% xylene – 100% ethanol – 95% ethanol – 80% ethanol

Washing
Phosphate buffered saline

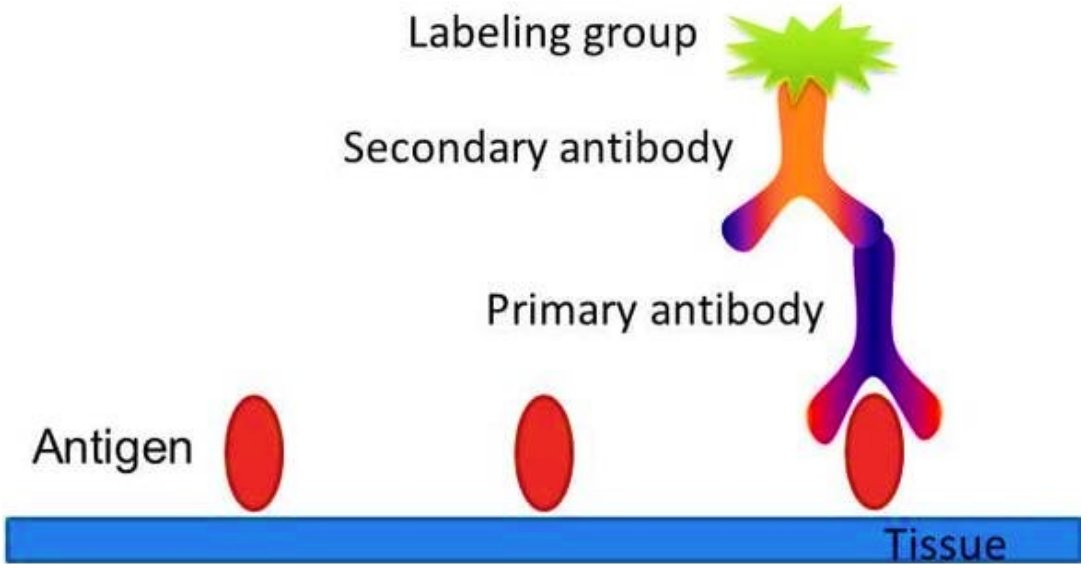
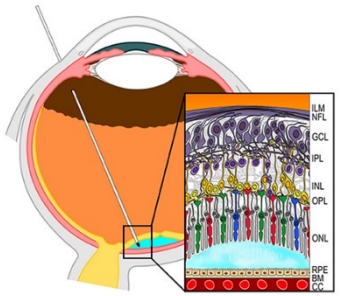


Fig.19 (Immunohistochemistry)
Source: CreativeBiomart

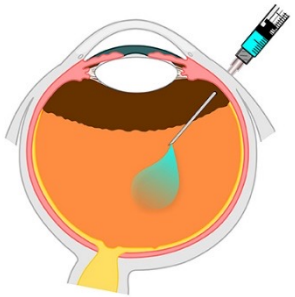
To show protein expression in the Retinal pigment Epithelium (RPE)

Two Type of Injection

Subretinal



Intravitreal



Remember …

8 LNPs with Different Characterization

4 Cholesterol

4 DSPC

Luciferase expression

apoE

Ai9

Mertk

C57BL6

The Effects of LNP Size and Cholesterol Modification Post-subretinal Injection

LNPs Kinetic were measured with Luciferase assay

↓

Luciferase activity was measurable at 4 hours post-injection

Increased to maximum level at 24 hours post-injection

Decreased by 48 hours post-injection

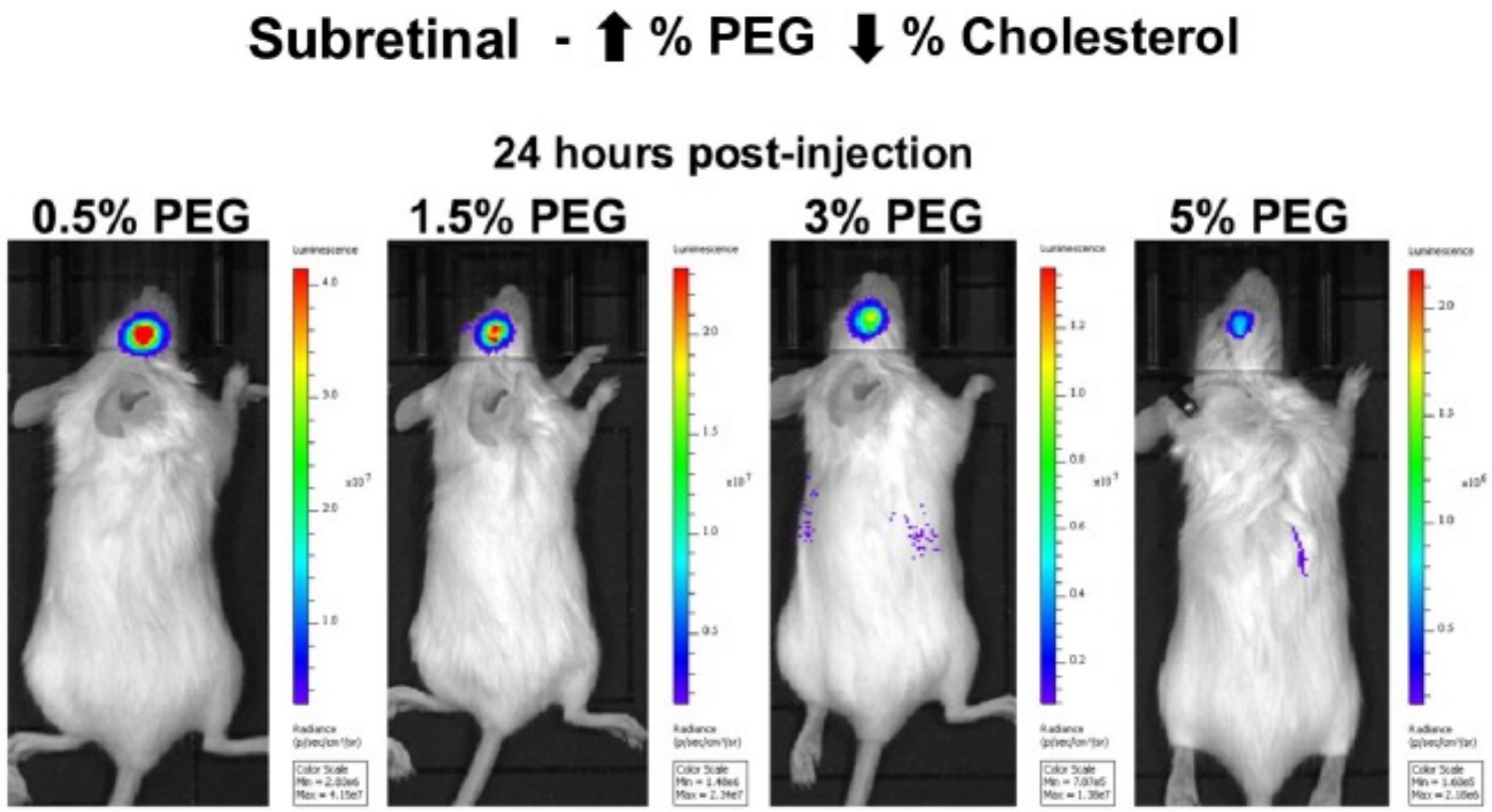


Fig.20 (Representative image demonstrated Luciferase activity in the eye 24 hours post-injection)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

The Effects of LNP Size and DSPC Modification Post-subretinal Injection

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↓

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Decreased by 48 hours post-injection

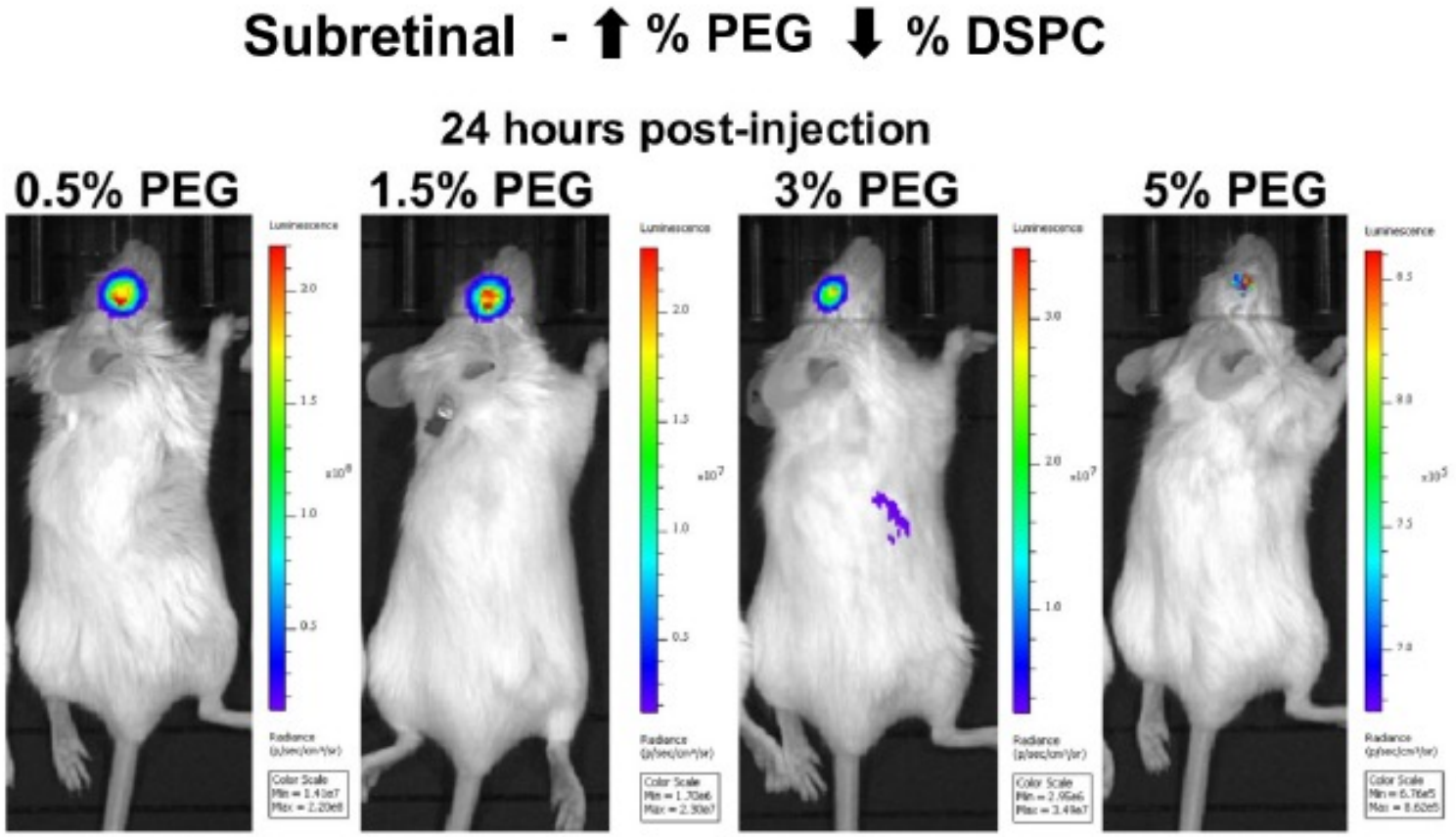

Fig.21 (Representative image demonstrated Luciferase activity in the eye 24 hours post-injection)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

Luciferase activity plotted for each group over time (Cholesterol)

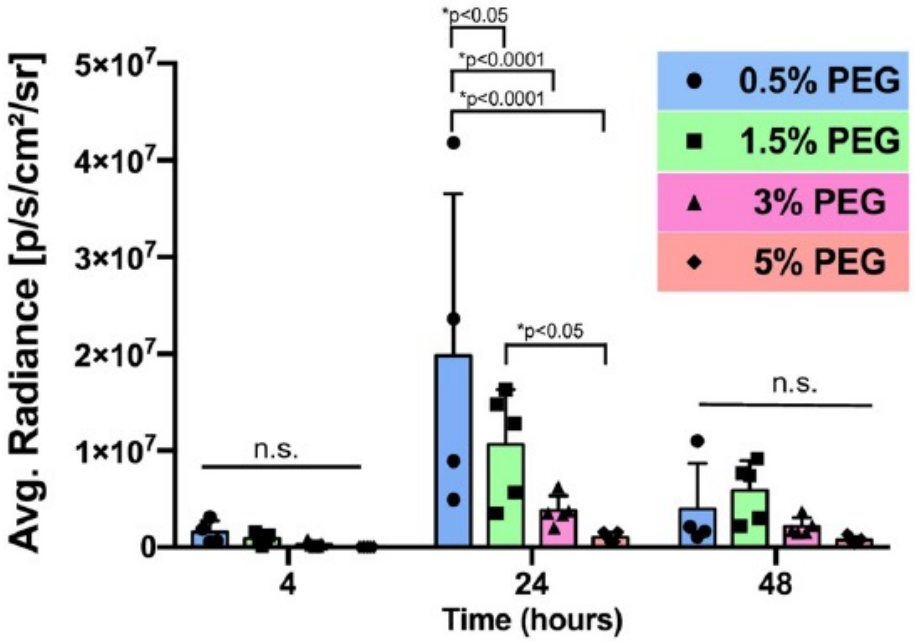

Fig.22(Luciferase activity plotted for each group over time Cholesterol)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

Luciferase activity plotted for each group over time (DSPC)

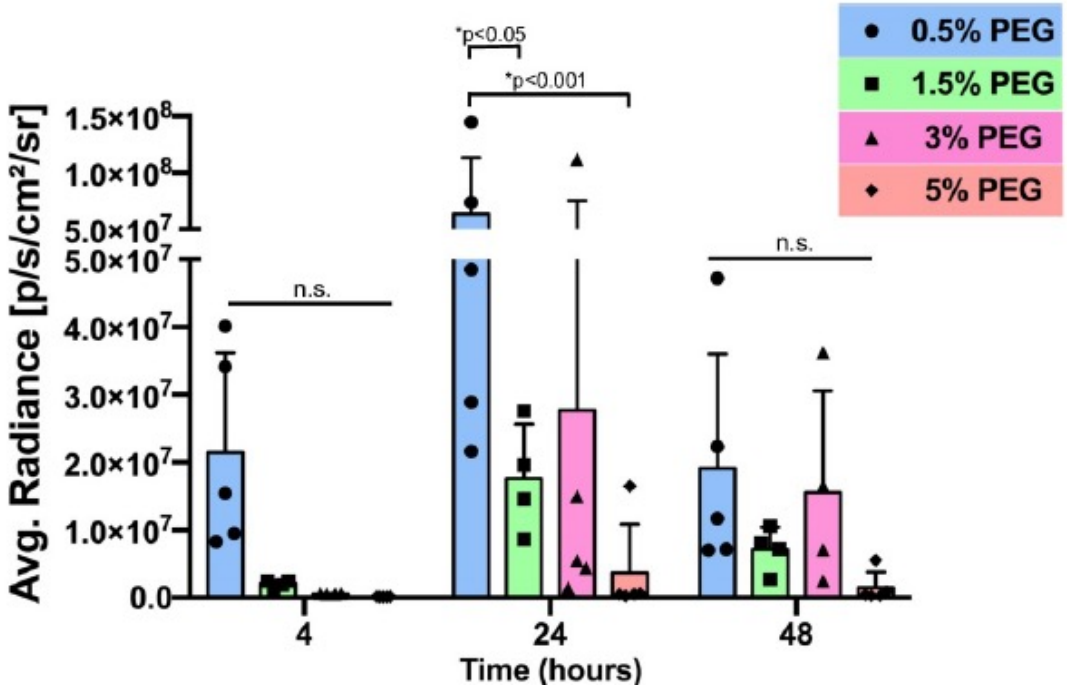

Fig.23 (Luciferase activity plotted for each group over time DSPC)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

Top Bright Field Bottom Fundus Images After 7 Days (Cholesterol)

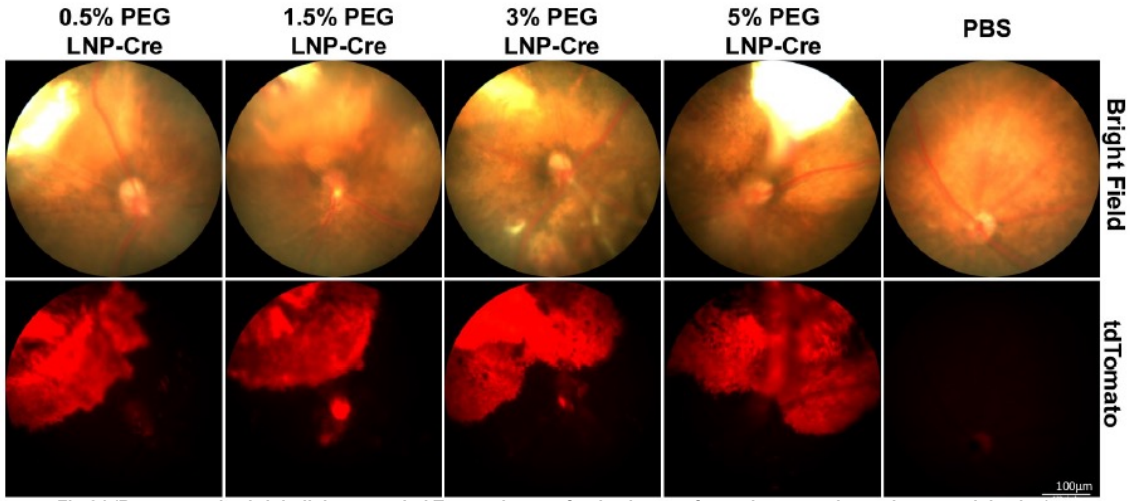

Fig.24 (Representative bright light top and td Tomato bottom fundus images for each group taken 7 days post-injection)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

Top Bright Field Bottom Fundus Images After 7 Days (DSPC)

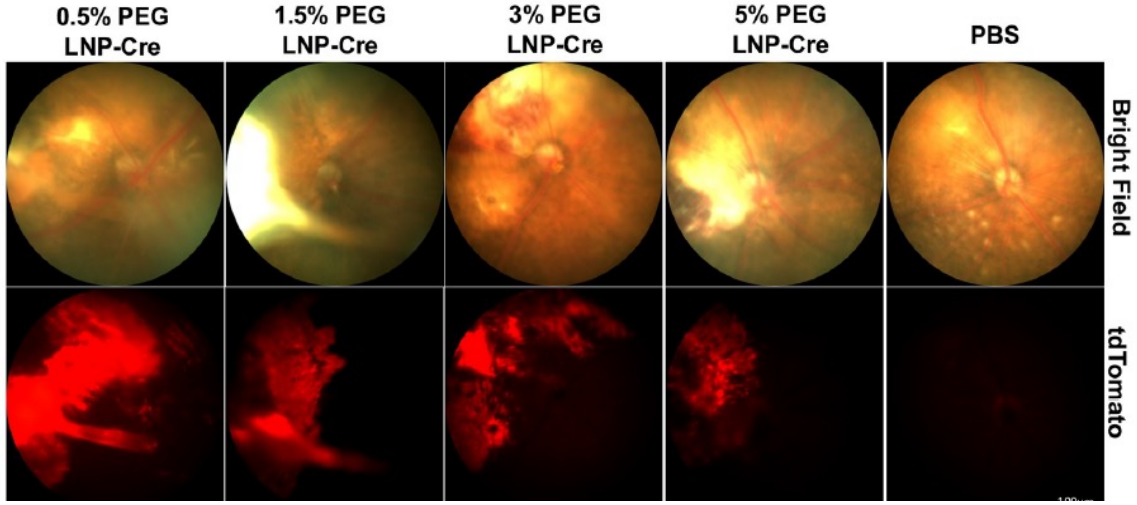

Fig.26 (Representative bright light top and td Tomato bottom fundus images for each group taken 7 days post-injection)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

Confocal Image of Immunohistochemistry (Cholesterol)

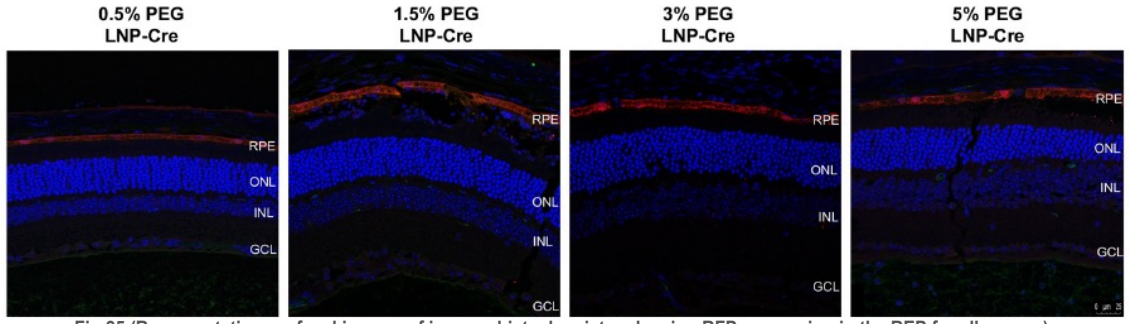

Fig.25 (Representative confocal images of immunohistochemistry showing RFP expression in the REP for all groups)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

Confocal Image of Immunohistochemistry (DSPC)

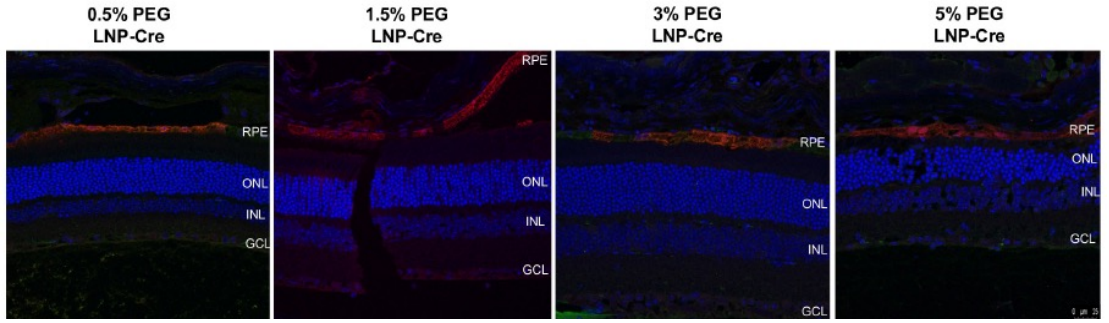

Fig.27 (Representative confocal images of immunohistochemistry showing RFP expression in the REP for all groups)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

The Effects of LNP Size and Cholesterol Modification Post-intravitreal Injection

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↓

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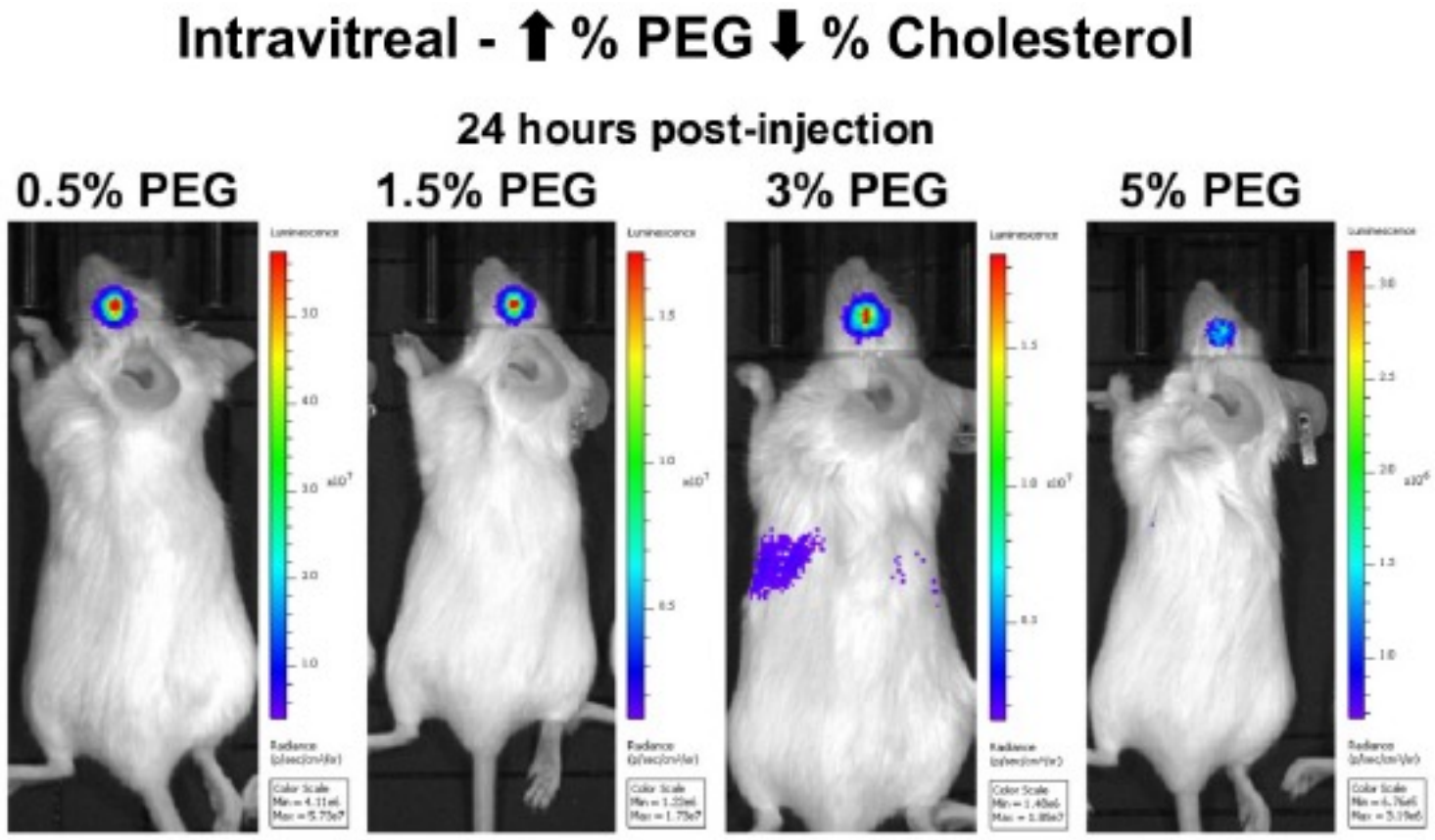

Fig.28 (Representative image demonstrated Luciferase activity in the eye 24 hours post-injection)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

The Effects of LNP Size and DSPC Modification Post-intravitreal Injection

LNPs Kinetic were measured with Luciferase assay

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Luciferase activity was measurable at 4 hours post-injection

Increased to maximum level at 24 hours post-injection

Decreased by 48 hours post-injection

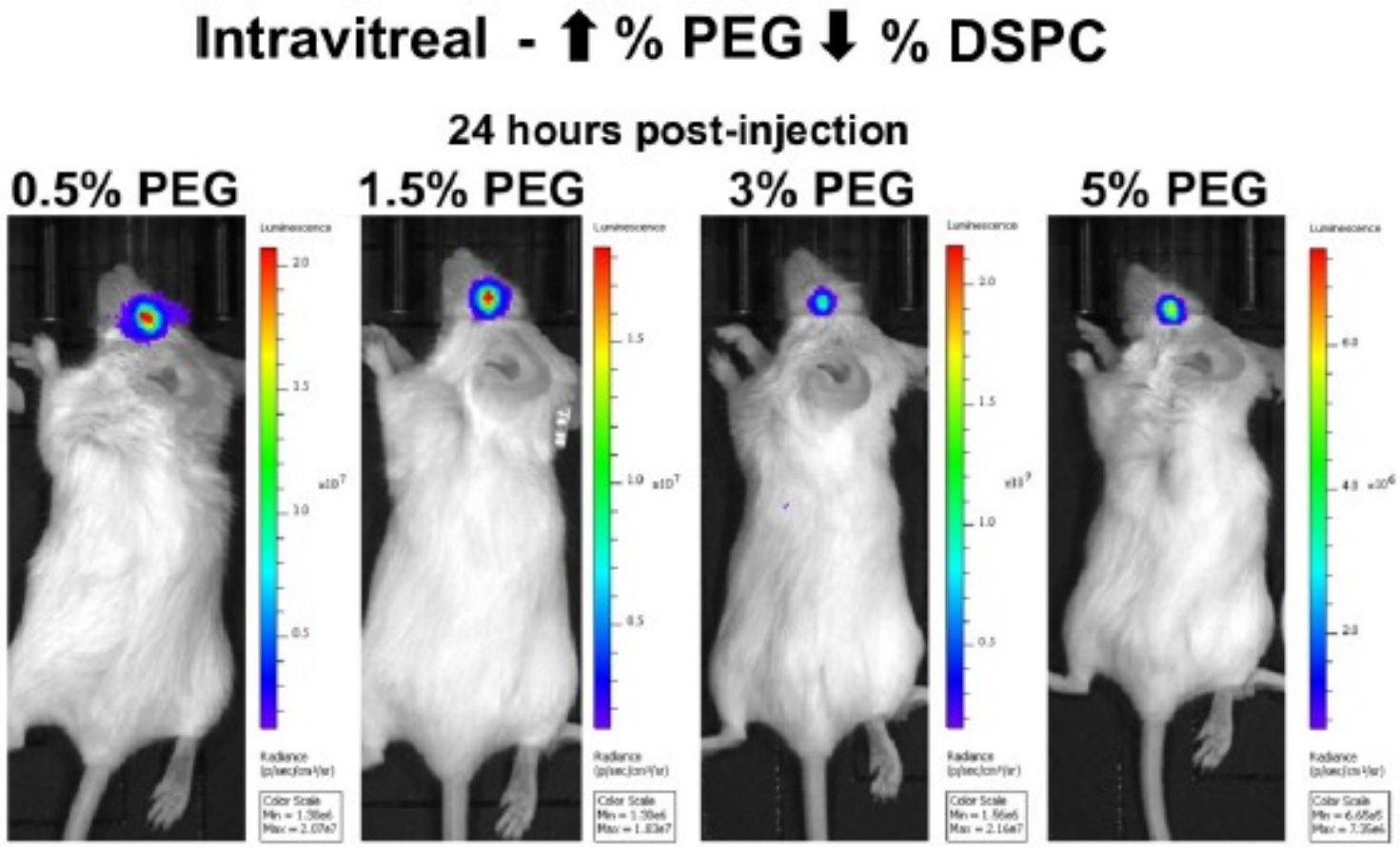

Fig.29 (Representative image demonstrated Luciferase activity in the eye 24 hours post-injection)

Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

Luciferase activity plotted for each group over time (Cholesterol)

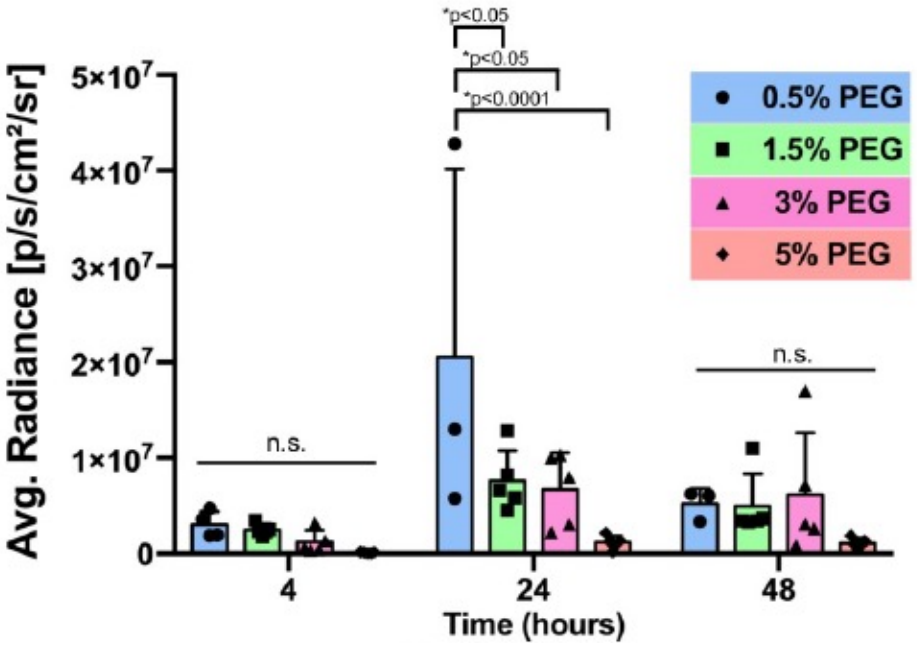

Fig.30(Luciferase activity plotted for each group over time Cholesterol)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

Luciferase activity plotted for each group over time (DSPC)

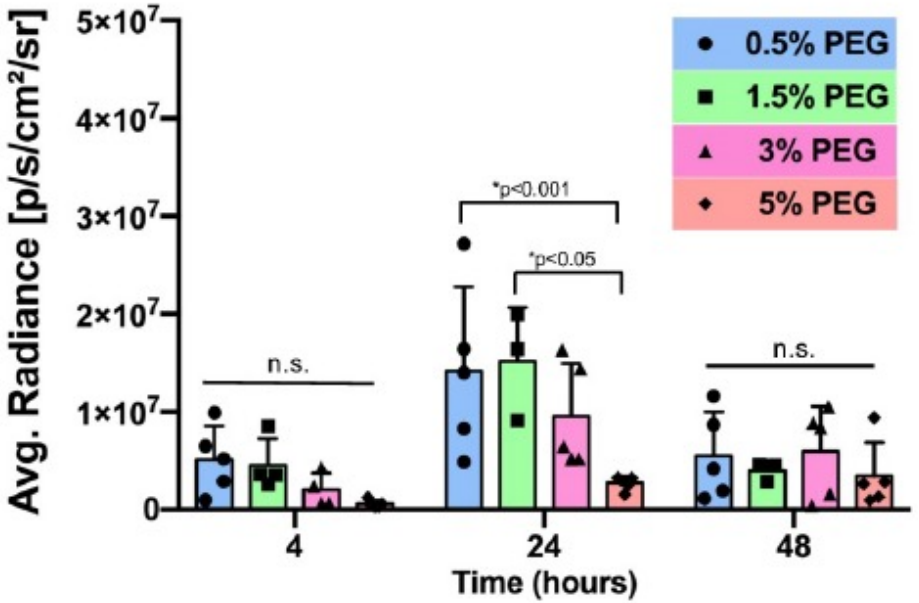

Fig.31 (Luciferase activity plotted for each group over time DSPC)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

Top Bright Field Bottom Fundus Images After 7 Days (Cholesterol)

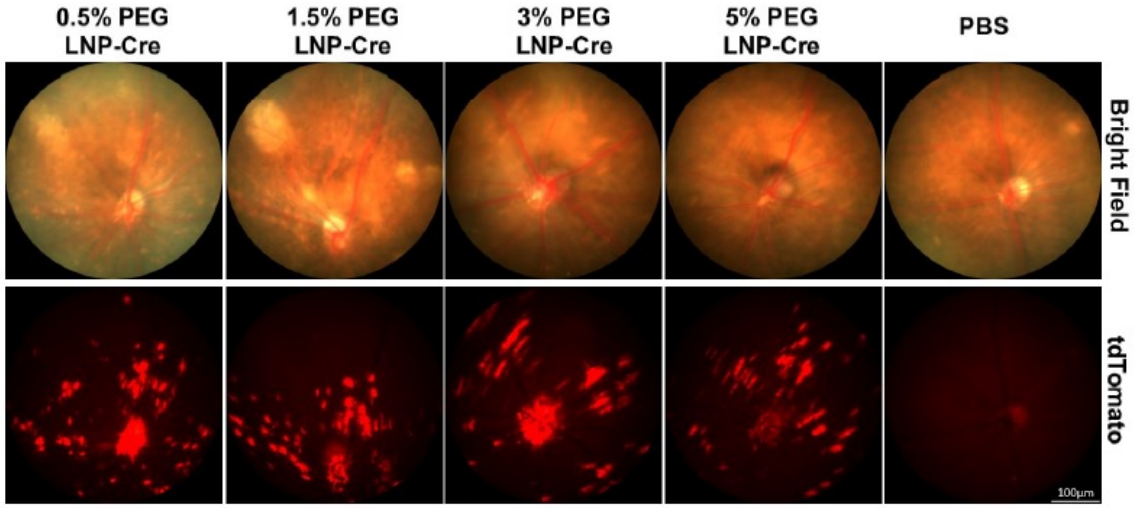

Fig.32 (Representative bright light top and td Tomato bottom fundus images for each group taken 7 days post-injection)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

Top Bright Field Bottom Fundus Images After 7 Days (DSPC)

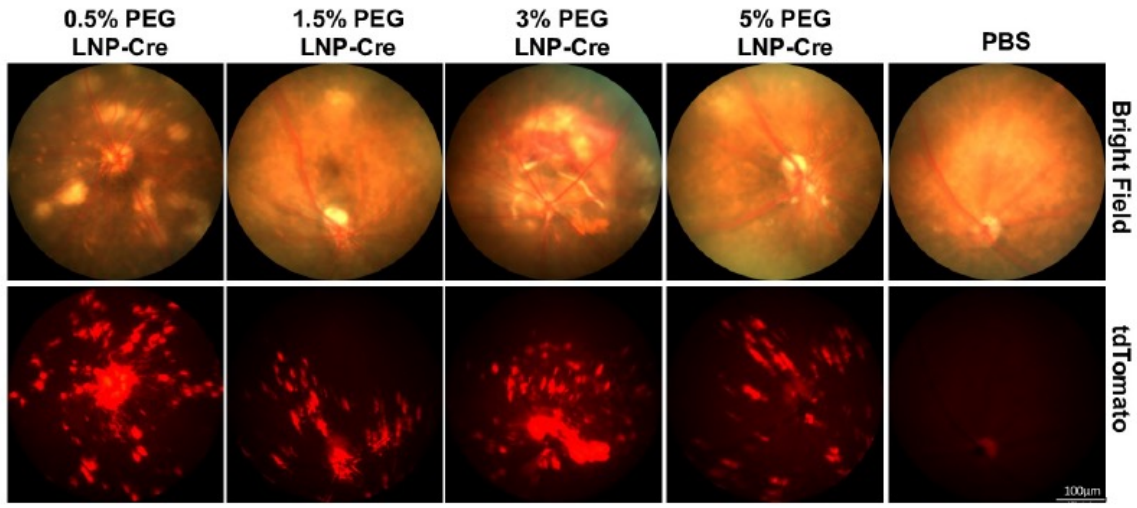

Fig.34 (Representative bright light top and td Tomato bottom fundus images for each group taken 7 days post-injection)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

Confocal Image of Immunohistochemistry (Cholesterol)

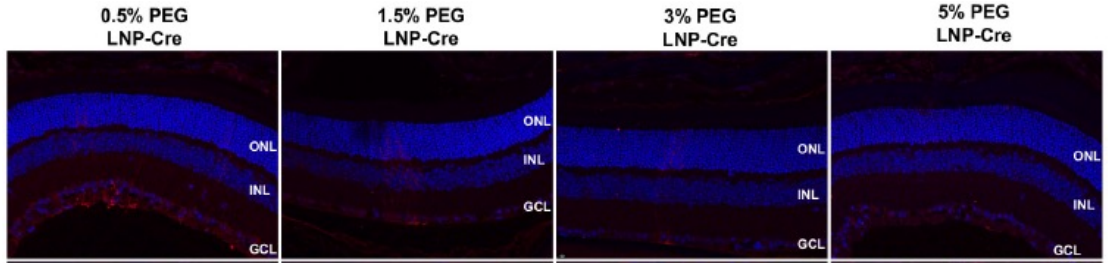

Fig.33 (Representative confocal images of immunohistochemistry showing RFP expression in the REP for all groups)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

Confocal Image of Immunohistochemistry (DSPC)

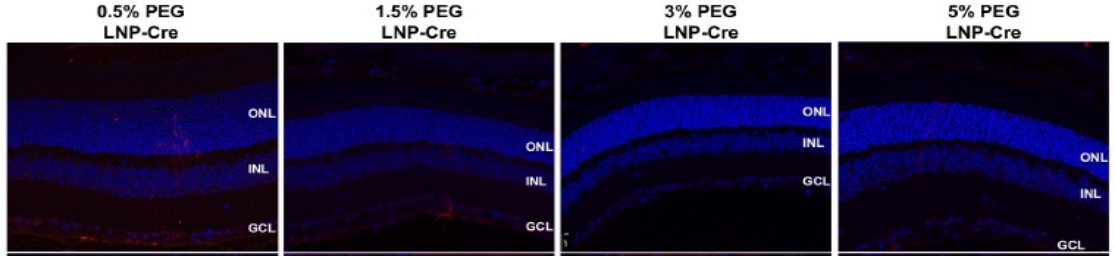

Fig.35 (Representative confocal images of immunohistochemistry showing RFP expression in the REP for all groups)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

To determine the location of protein expression in the eye

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Luciferase mRNA + Cre mRNA was injected subretinally to Ai9 Mice

↓

What do you expect?

Ai9 Mice

Prevent transcription of red fluorescent protein variant td Tomato

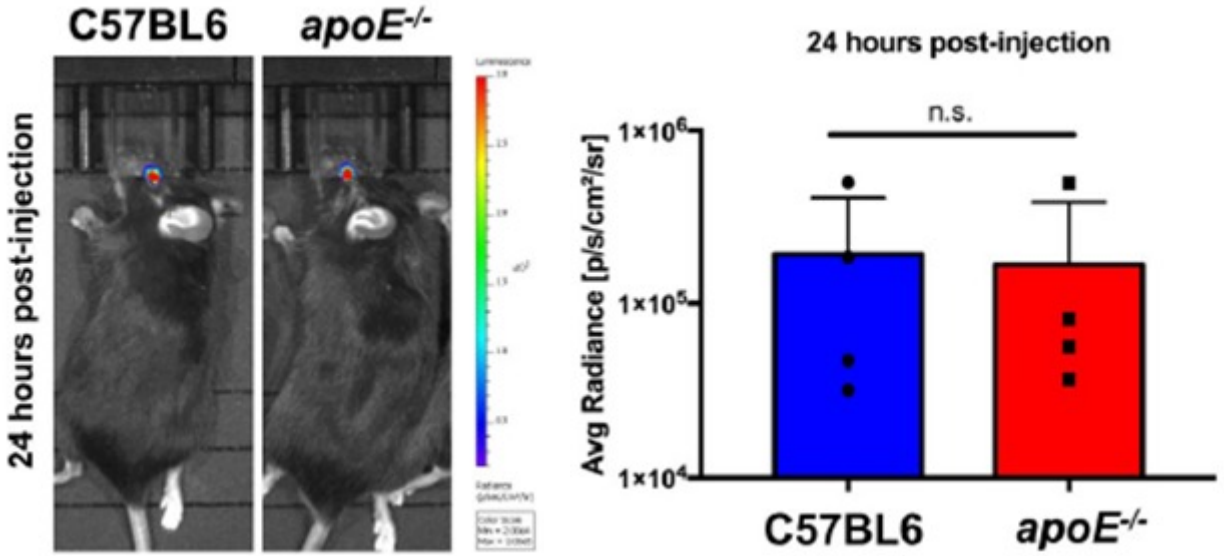

Fig.36 (Representative image demonstrating Luciferase activity in C57BL6 and apoE eyes 24 hours post-injection)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

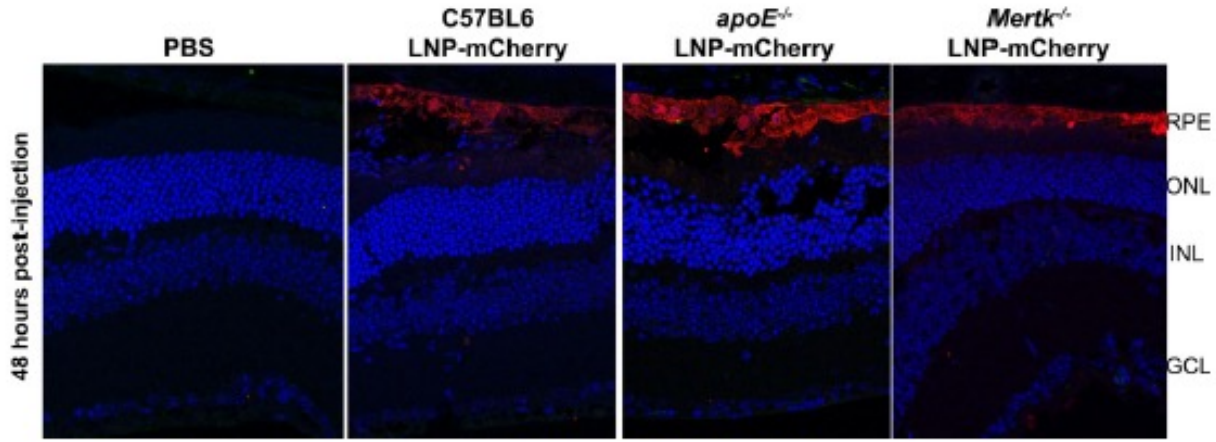

Fig.37 (Representative confocal images of immunohistochemistry showing mCherry expression in the REP)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

To verifying that neither neither phagocytosis nor apoE adsorption are responsible for LNP intracellular delivery the RPE

Conclusion

- The particles with less PEG (0.5%) and larger in size (150nm) facilities the highest level of protein expression post-subretinal and intravitreal delivery

- LNPs can transfect Muller glia, the ONH and the TM post-intravitreal delivery which could have significant impacts for retinal degeneration and glaucoma

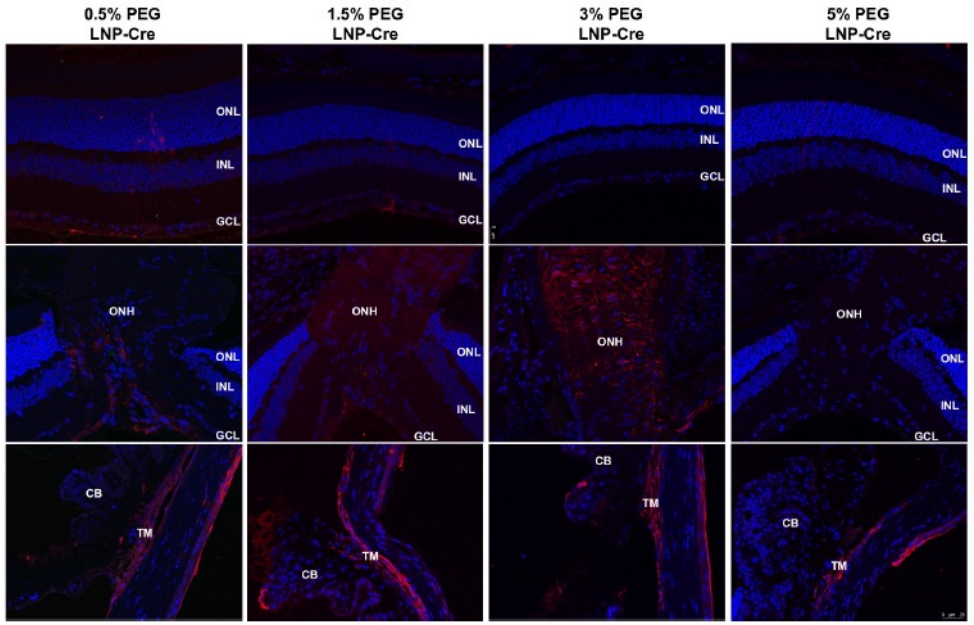

Fig.38 (Representative confocal images of immunohistochemistry showing RFP expression in the REP for all groups)
Source: Research Article The Effects of PEGylation on LNP Based mRNA Delivery to the Eye

What is the different between LNPs approach and viral delivery system for gene therapy?

References

- The effects of PEGylation on LNP based mRNA delivery to the eye by Renee C. Ryals, Siddharth Patel, Chris Acosta, Madison McKinney, Mark E. Pennesi and Gaurav Sahay. Health & Science Unvirsity, Portland. Department of Pharmaceutical Sciences, United State of America.
  October 29, 2020