

Extended Release IOP-Lowering Formulation

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INTRODUCTION

- The leading cause of irreversible blindness in the world is glaucoma and 90% of all glaucoma cases present as primary open angle glaucoma (POAG). Elevated intraocular pressure (IOP) is one of the most significant risk factors of visual field loss in POAG, therefore IOP reduction is the first-line therapeutic option. Unfortunately, current medications do not address underlying pathologies or genetic variations.
- By combining a forward murine genetics approach with cell biology, pharmacology and analysis of human GWAS data, we identified a genetic locus that significantly modulates IOP, namely the calcium channel, voltage-dependent, $\alpha 2\delta 1$ subunit (aka *Cacna2d1*). We demonstrated that pregabalin (PRG), a molecule with high affinity and specificity for CACNA2D1, lowers IOP in a dose dependent manner. However, the duration of the effect was limited and IOP returned to baseline by 6 hours after dosing.
- Our goal in this study was to improve the efficacy of PRG by incorporating it in a biocompatible topical formulation that has increased mucoadhesive properties, sustained corneal permeability, miniscule particle size, penetration enhancing ability, sustained release behavior and a clear final formulation.

DESIGN & METHODS

Preparation of PRG multiple W/O/W microemulsion (ME) eye drops

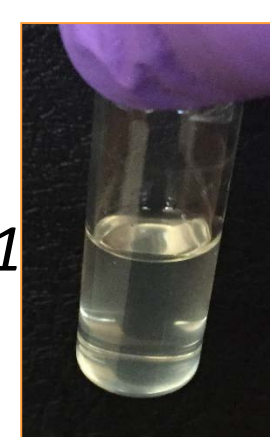
- Construction of ternary and pseudo-ternary phase diagrams and preparation of the primary W/O ME
- Preparation of external aqueous phase and incorporation of the bioadhesive polymers

In vitro evaluations of our PRG ME eye drops

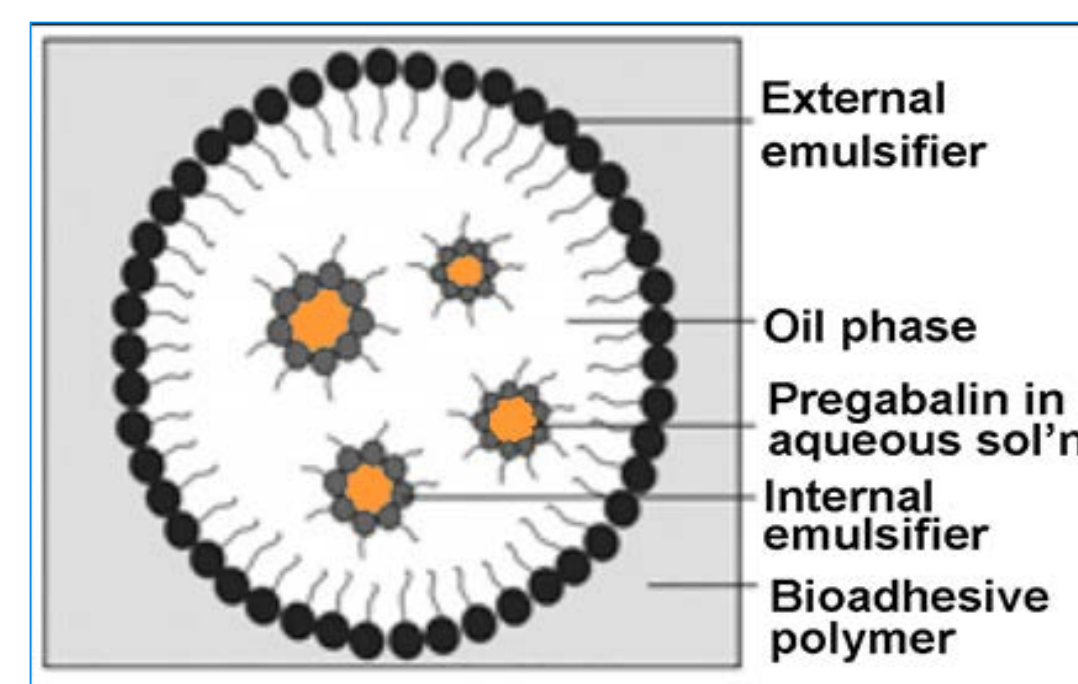
- Measurement of droplet size, polydispersity index (PDI) and zeta potential
- TEM examination of ME
- Determination of cumulative amount of PRG released from ME
- Determination of viscosity and bioadhesive force
- Cytotoxicity study using MTT assay

In vivo evaluations of the safety and IOP-lowering efficacy of our formulations using Dutch belted rabbits

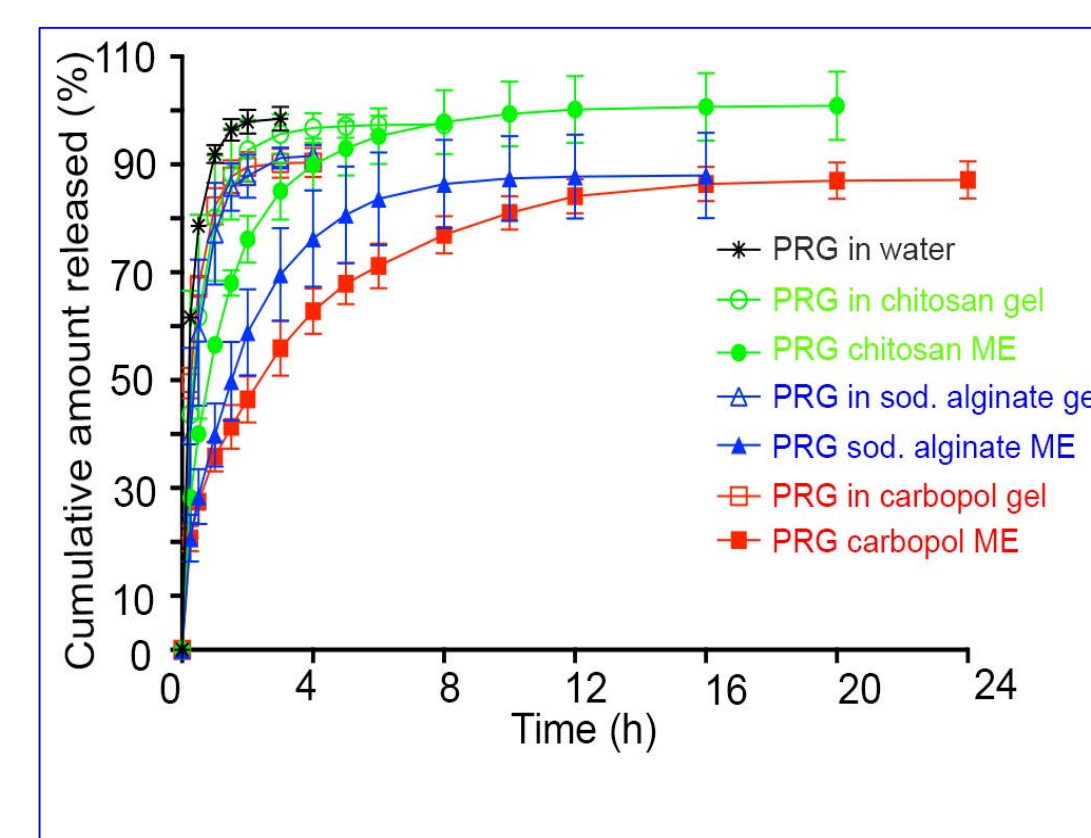
- Calculation of pharmacodynamic parameters using a single dose design
- DB rabbits
- Mice with B or D haplotypes of *Cacna2d1*
- Slit lamp examinations
- Systemic BP measurements



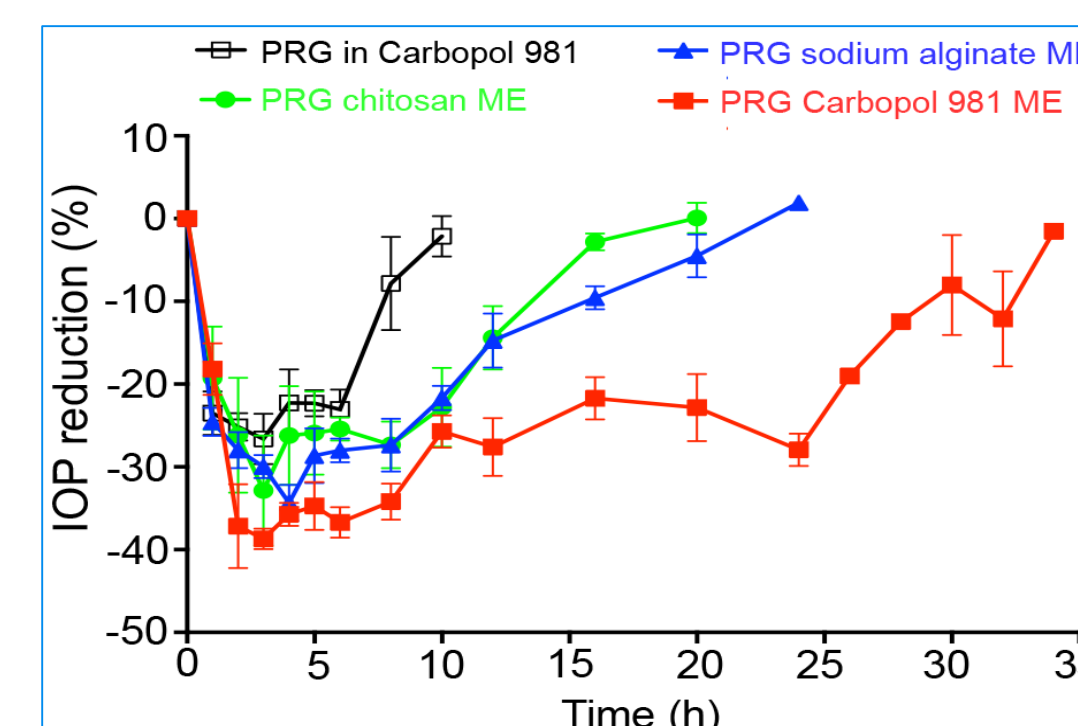
RESULTS



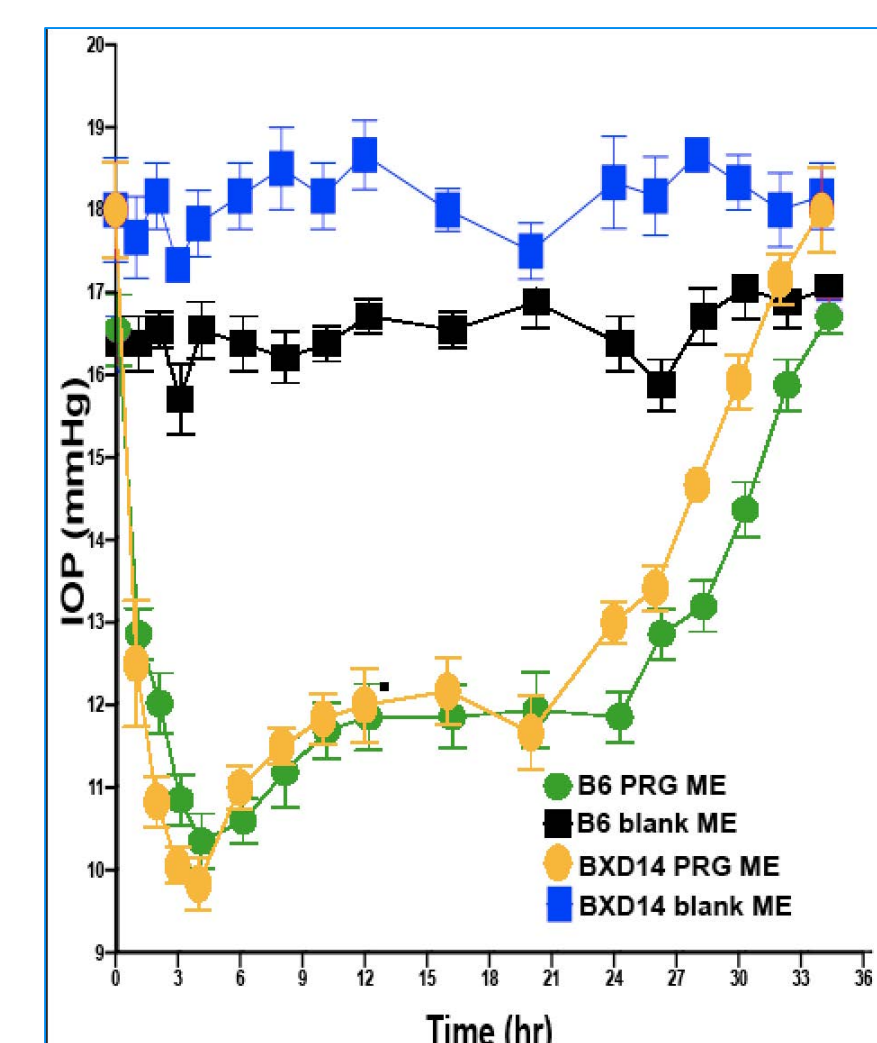
- Cartoon showing that PRG is incorporated in the inner layer of the formulation



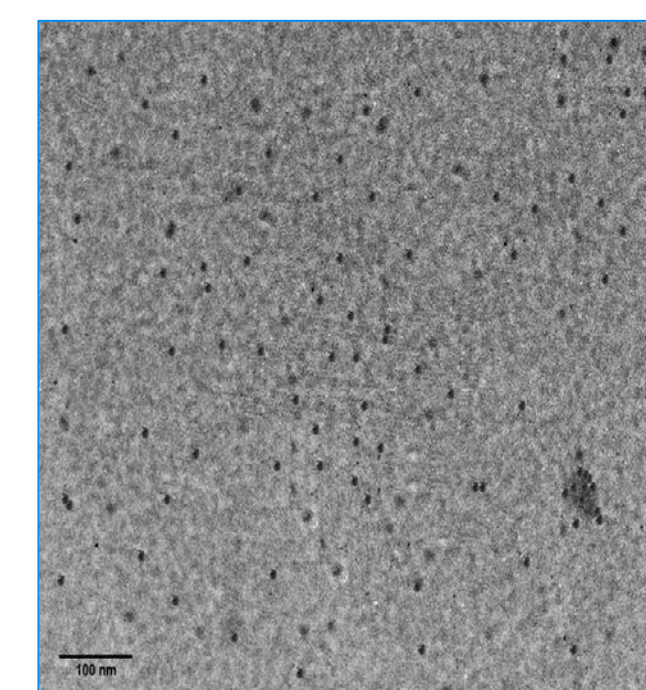
- ME formulations sustain release of PRG for up to 24h



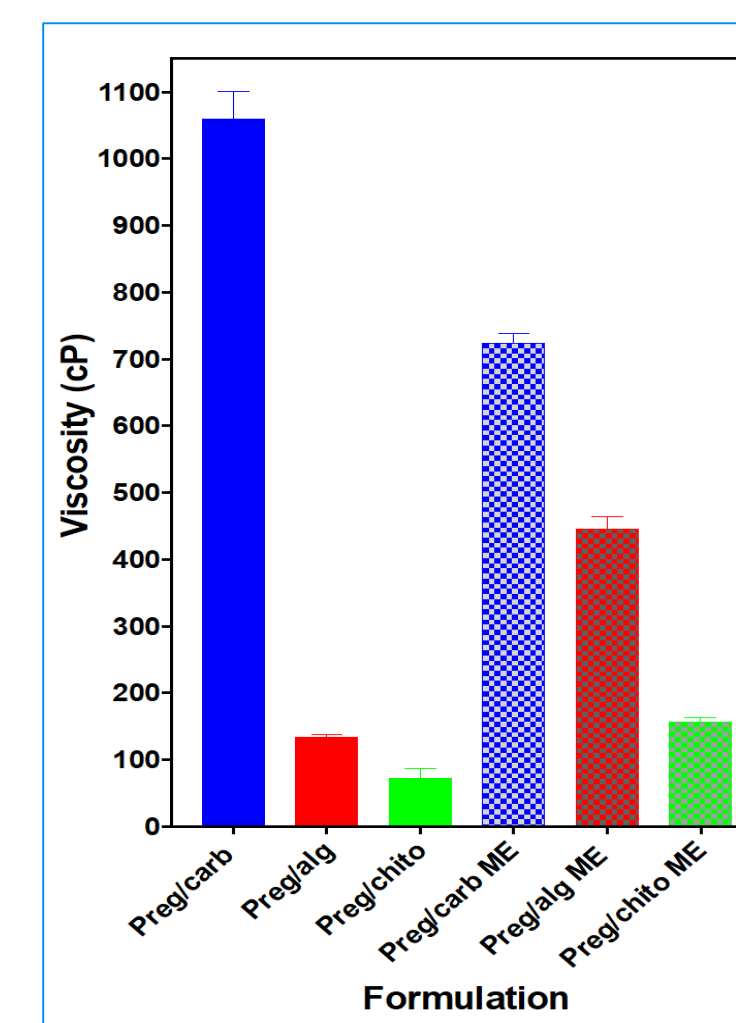
- Inclusion of PRG in our ME greatly increases its efficacy after a single dose is applied to the cornea of Dutch belted rabbits



- Our PRG ME lowers IOP by ~30% in mice with the B haplotype of *Cacna2d1*



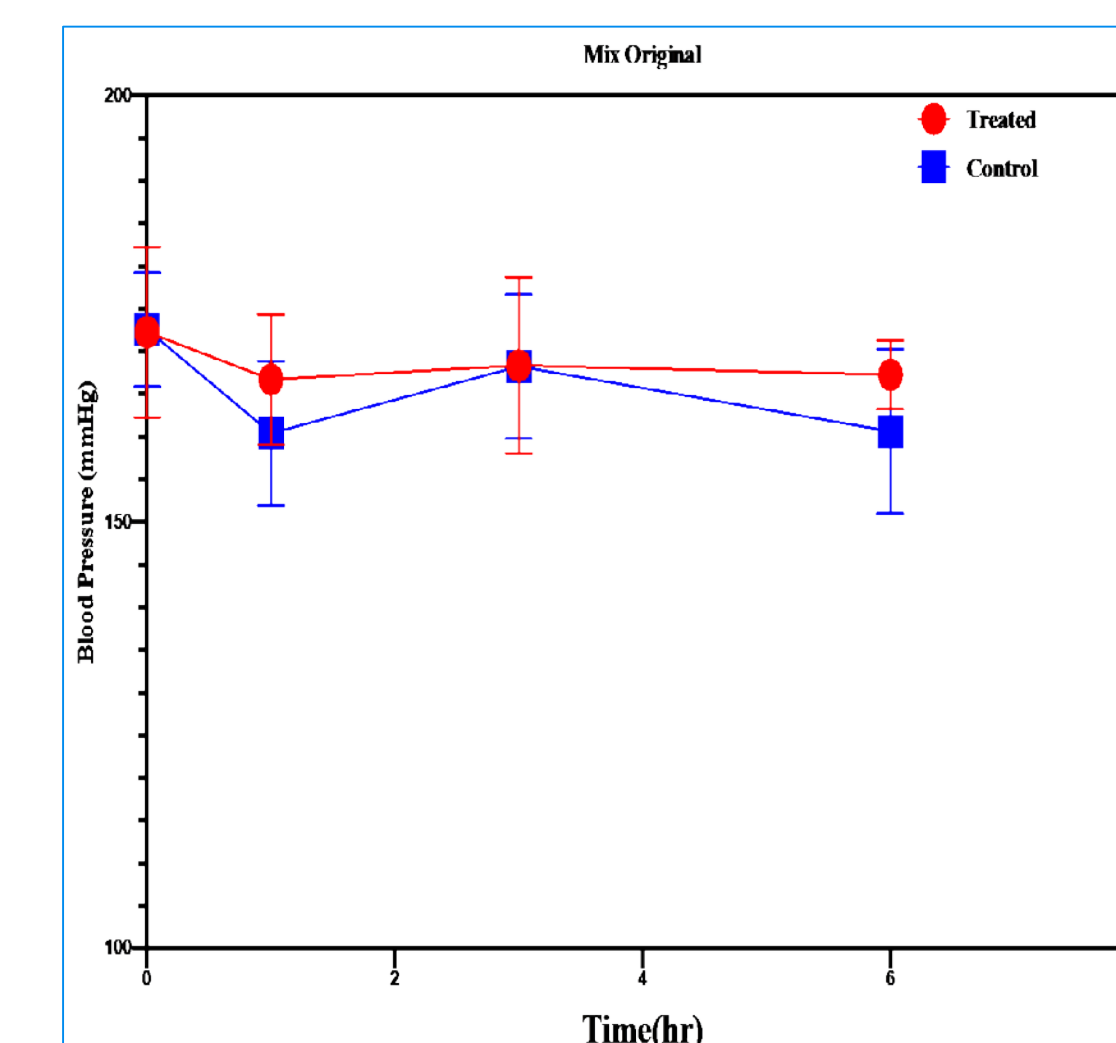
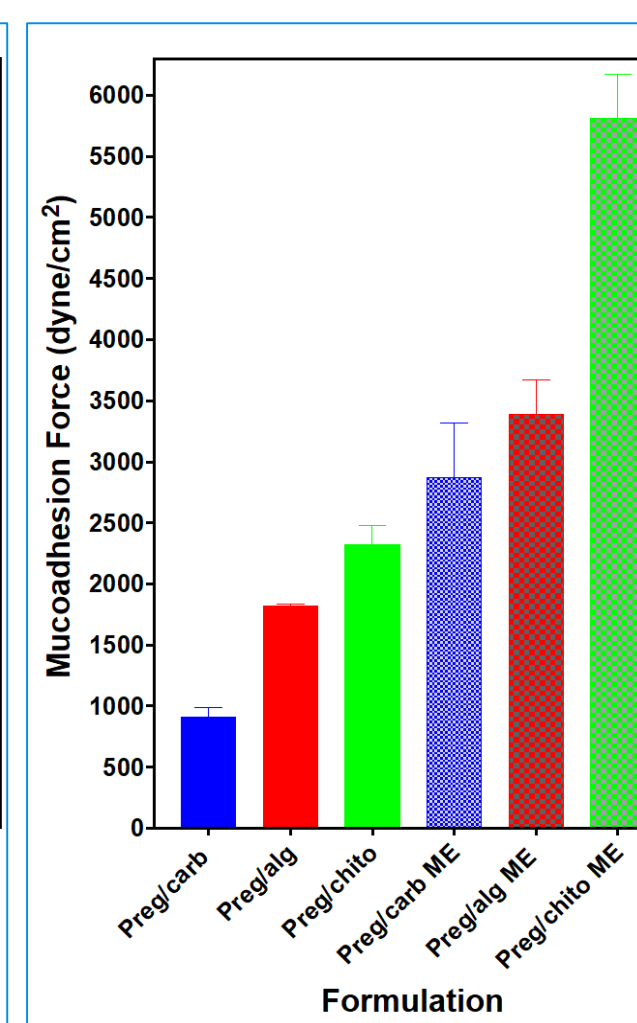
- Spherical ME globules by TEM



- Carbopol ME has high viscosity, while chitosan ME has the highest bioadhesive force

Formulation	Droplet size (nm)		PDI		Zeta potential (mV)	
	Blank	Medicated	Blank	Medicated	Blank	Medicated
PRG in ME-1	17.4±0.0	17.4±0.3	0.36±0.0	0.37±0.0	15.9±3.3	10.2±2.3
PRG in ME-2	16.8±0.3	16.5±0.2	0.34±0.0	0.34±0.0	-26.8±0.8	-26.7±1.7
PRG in ME-3	16.0±0.2	15.4±0.1	0.26±0.0	0.26±0.0	-30.1±2.2	-26.3±1.0

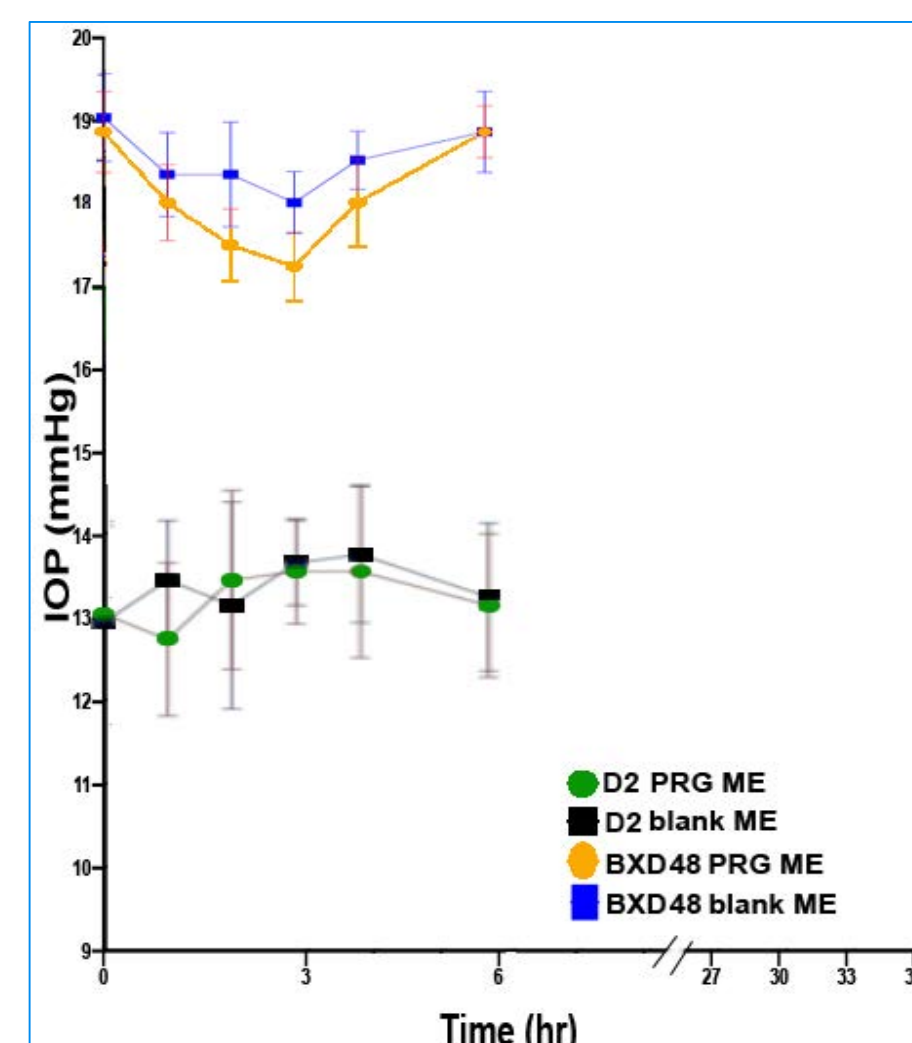
- ME formulations have uniform very small size (<20nm) and a high zeta potential that will keep it stable



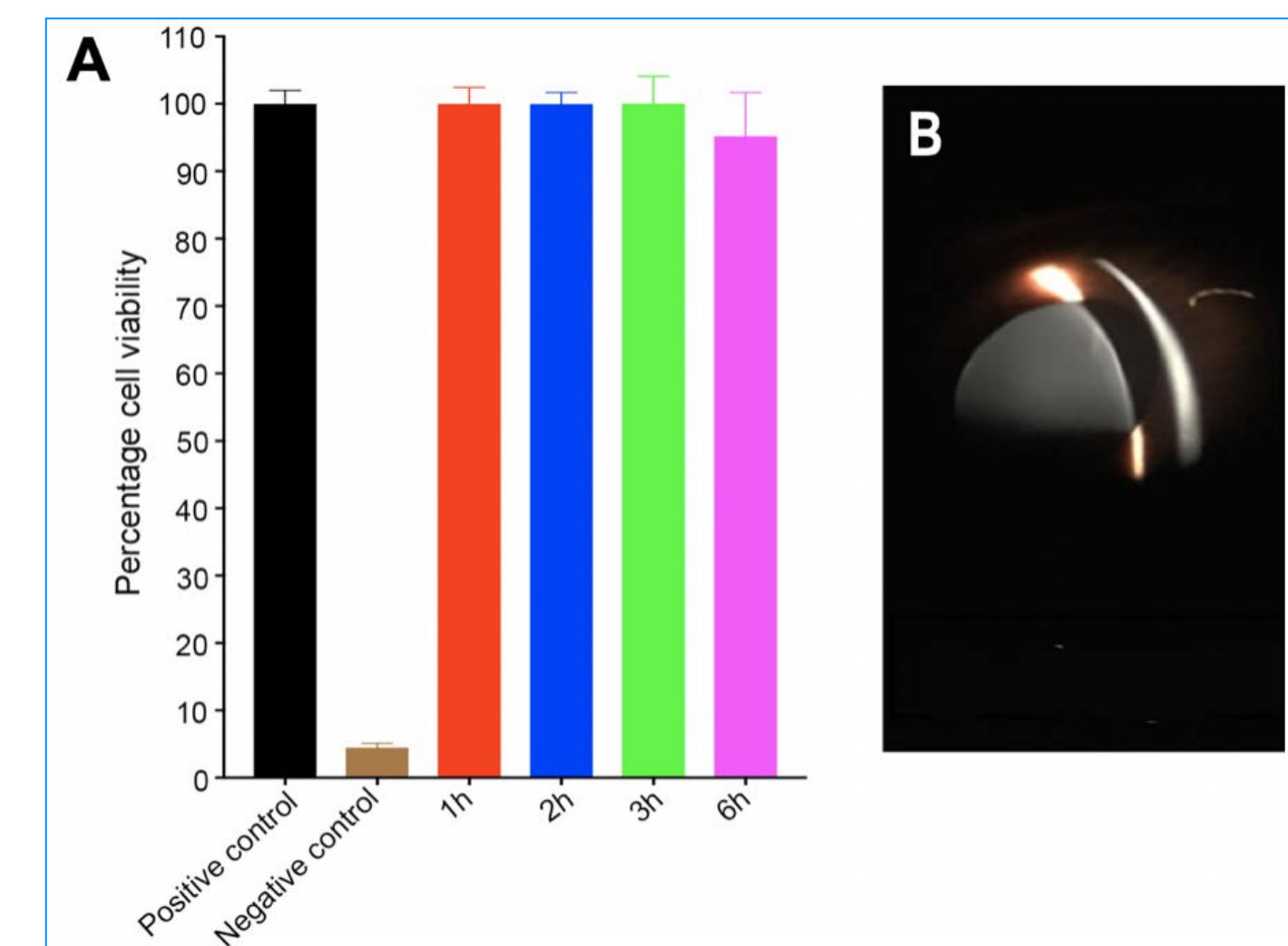
- Carbopol ME does not elevate systemic BP after topical dosing

Pharmacodynamic parameters	Ophthalmic Eye Drops			
	PRG chitosan ME	PRG sodium alginate ME	PRG Carbopol ME	PRG in Carbopol
Baseline IOP	21.2±0.4	20.9±0.2	21.4±0.7	22.1±0.4
IOP at T _{max}	13.9±1.0	13.6±0.2	12.3±0.5	15.7±0.4
ΔIOP	-7.3±1.3	-7.3±0.4	-9.1±0.8	-6.5±0.4
% Reduction in IOP	34.1±5.4	35.0±1.7	42.3±2.6	29.4±1.4
T _{max} (h)	4.0±1.0	3.7±0.3	3.3±0.9	3.3±1.5
T _{end} (h)	20.0±0.0	24.0±0.0	32.7±1.3	9.3±0.7
AUC _{total} (% · h)	69.5±14.2	80.7±4.6	169.9±13.4	39.0±4.0

- PRG in the Carbopol ME provides the greatest IOP-lowering efficacy in Dutch belted rabbits. IOP is lowered by ~40% and doesn't return to baseline for 34 hours



- Our PRG ME is not as effective in mice with the D haplotype of *Cacna2d1*



- Our PRG ME is safe to a corneal epithelial limbal stem cell line by MTT assay (A) and to the cornea by slit lamp biomicroscopy (B)

CONCLUSIONS

- In the current study, we develop a once-daily topical PRG-loaded ophthalmic formulation
- Our formulation components were carefully selected to be highly biocompatible, bioadhesive, and provide continuous PRG release for up to 24h
- Because of its miniscule particle size (<20nm), our ME is transparent and should not blur vision
- TEM examination proved that the ME globules are spherical in shape and uniformly distributed
- The Carbopol ME sustains release of PRG for up to 24 hours
- The viscosity and mucoadhesive forces of our formulations should support a sustained presence of formulation on the eye
- Our PRG Carbopol ME does not increase the systemic BP after topical dosing
- Our PRG Carbopol ME supports the largest decrease in IOP (40%) for the longest time (34 hours)
- The haplotype of *Cacna2d1* influences the magnitude of the IOP-lowering properties of our PRG Carbopol ME
- Our formulations are non-toxic, as illustrated by slit-lamp biomicroscopic exams and *in vitro* cytotoxicity studies
- Our *in vivo* study using Dutch belted rabbits demonstrates that our formulations markedly enhance the efficacy and prolong the duration of the IOP-lowering effect of PRG
- Our ME is a promising carrier that sustains the release and prolongs the duration of action of PRG. It is also compatible with any other water-soluble drugs.

NEXT STEPS

The next steps in this study include:

- Determination of formulation stability
- Repeat study using a multi-dosing paradigm to evaluate tachyphylaxis and safety
- Determination of mechanism of action by which PRG lowers IOP

ACKNOWLEDGEMENTS

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