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**Polymeric nanoparticles augment
the ocular hypotensive effect
of melatonin in rabbits**

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Alternative strategies for eye treatment

- ⇒ bioadhesive hydrogels
- ⇒ *in situ* gel forming biomaterials
- ⇒ vesicular nanocarriers
- ⇒ polymeric nanoparticles
- ⇒ lipid-based nanocarriers (NLC, SLN)
- ⇒ inserts
- ⇒ cyclodextrins
- ⇒ micro/nano-emulsions
- ⇒ high-viscosity fluids

Advantages of ocular DDS

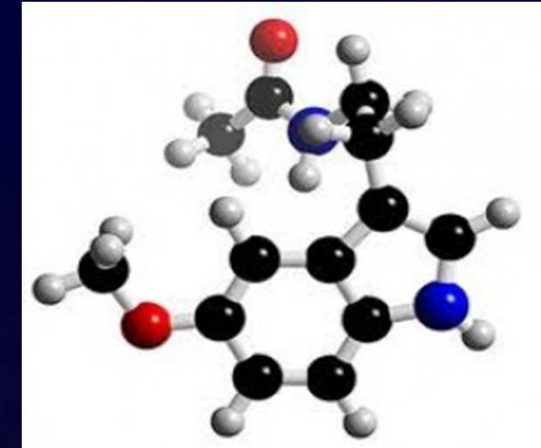
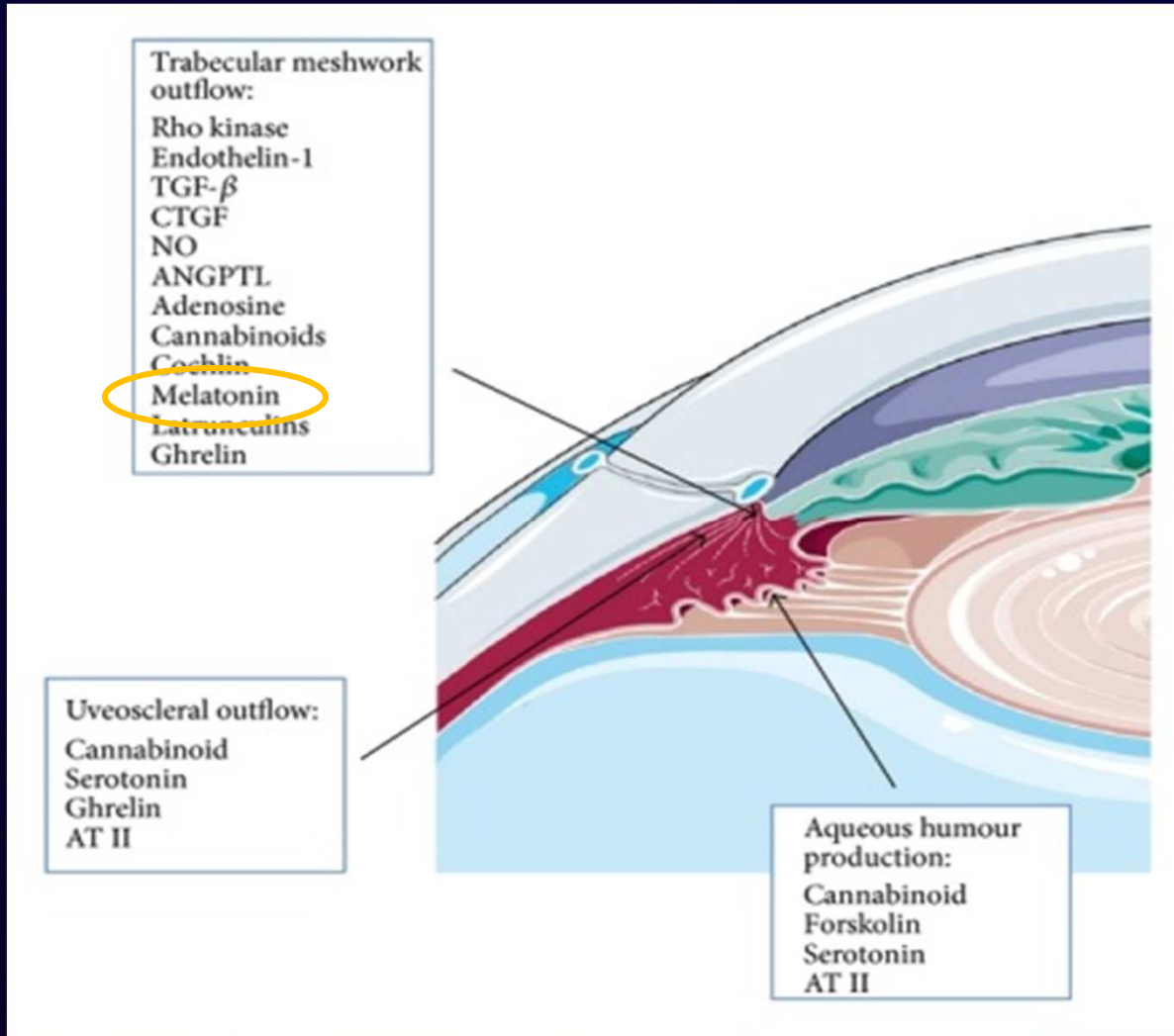
- ✓ **Mucoadhesion**
- ✓ **Prolonged drug release**
- ✓ **Enhanced drug absorption**
- ✓ **Side-effects reduction**
- ✓ **Ocular clearance lowering**

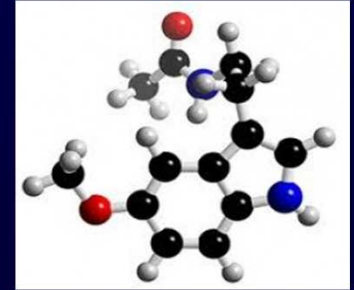


**The new NANO-*i* Research Center
is proposing to perform
basic and industry-oriented researches
in the field of controlled/targeted
ocular drug delivery**



new therapeutic targets for glaucoma



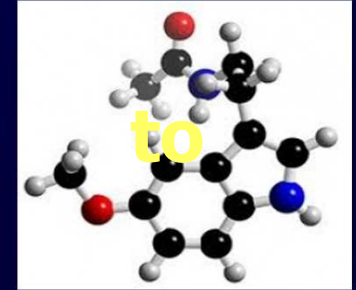


MEL receptors MT_1 and MT_2 are distributed in the cornea, choroid, sclera, photoreceptors, RGCs and retinal blood vessels.

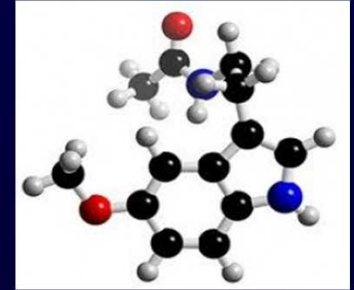
MT_1 receptors have been identified in the corneal epithelium, stroma, sclera, and endothelium of *Xenopus* eyes.

Three types of melatonin receptors, namely Mel_{1a} (MT_1), Mel_{1b} (MT_2) and Mel_{1c} are localized in the retina.

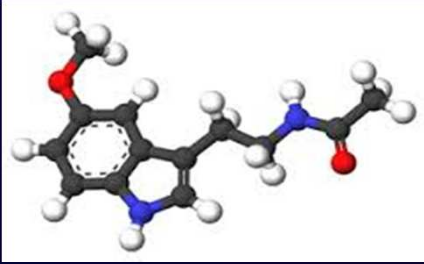
In the eye, locally synthesized melatonin has been associated different actions:



- regulate retinomotor movements
- rod outer segment disc shedding
- dopamine synthesis and release
- differential regulation of the growth and remodeling of fibrous and cartilaginous scleral layers
- aqueous humor secretion &
- circadian control of IOP
- antioxidant effect (free radical scavenger)
- protect photoreceptor outer segment membranes from light-induced free radical attack.



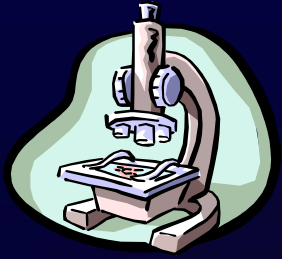
- **A study revealed that KO mice for MT1 receptors had higher IOP levels during the nocturnal hours than controls or KO mice for MT2 receptors at 3 and 12 months of age.**
- **Administration of exogenous melatonin significantly reduced IOP levels in wild-type mice, but not in the MT1 knock-out mice.**



the studied nanocarriers:

**PLGA & PLGA-PEG
NANOPARTICLES**

**SOLID LIPID
NANOPARTICLES
(SLN)**



PLGA & PLGA-PEG NANOPARTICLES

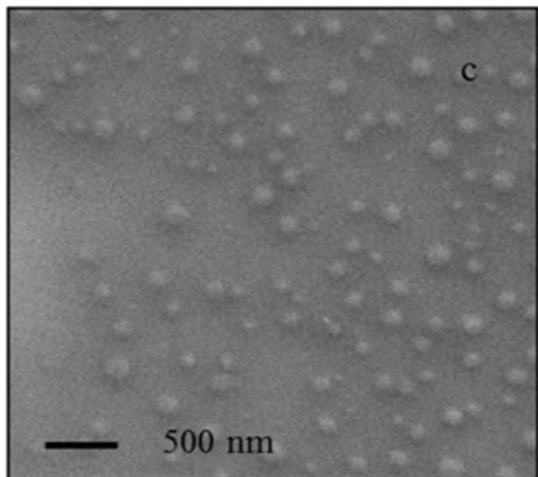
were produced using a
solvent displacement technique
at various MEL concs. (1, 3, 5% by weight)

mean size: **50-300 nm**

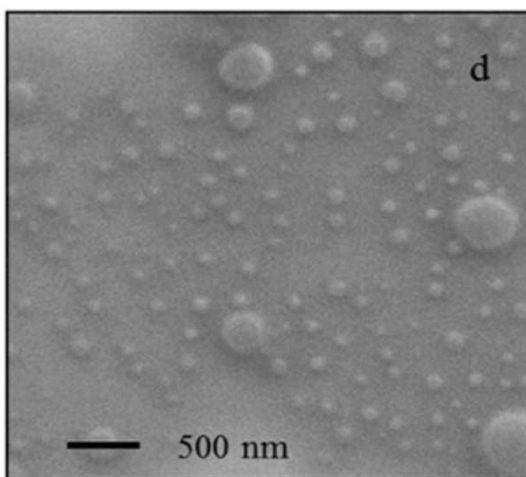
Zeta potential: -35 mV / -8 mV

possibility of - freeze-drying
- sterilization

Before freeze-drying



After freeze-drying



SEM micrographs of RGP-MEL1 before (a) or after freeze-drying (b)

(0.5% glucose)

**PLGA &
PLGA-PEG
NP**

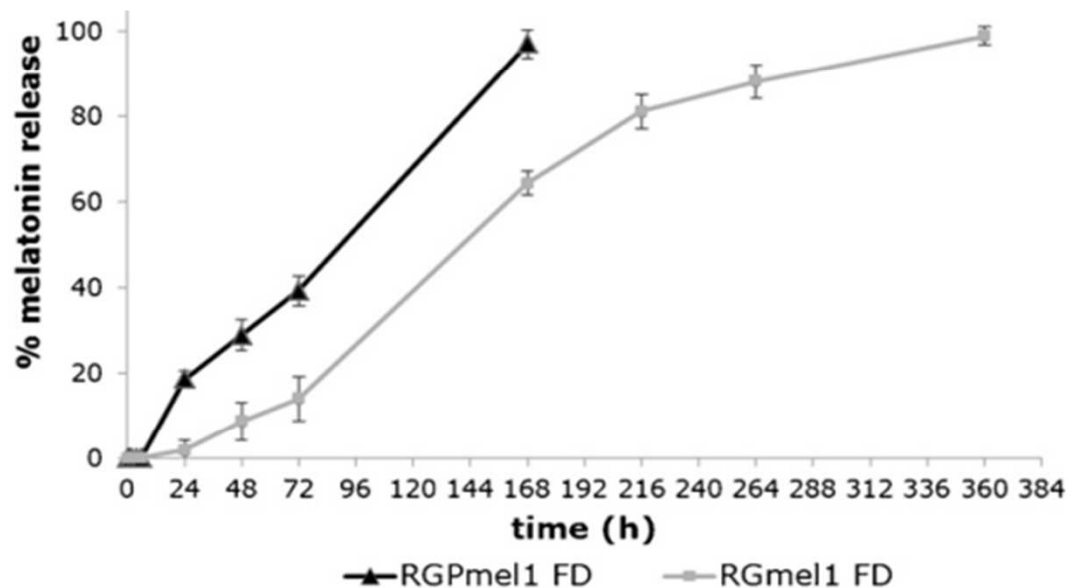
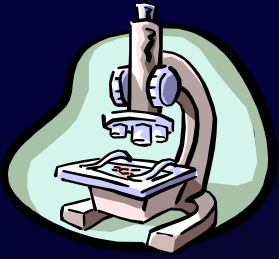


Fig. 5. *In vitro* release profiles of melatonin from NPs. Experiments were carried out at 37 ± 0.5 °C in isotonic phosphate buffer solution (PBS, pH 7.4), under constant stirring. Each point represents the mean value of five different experiments \pm SD.



SLN

by a lab-developed QESD technique
(Quasi-emulsion Solvent Diffusion)

formulation variables: DDAB,
palmitic or stearic acids

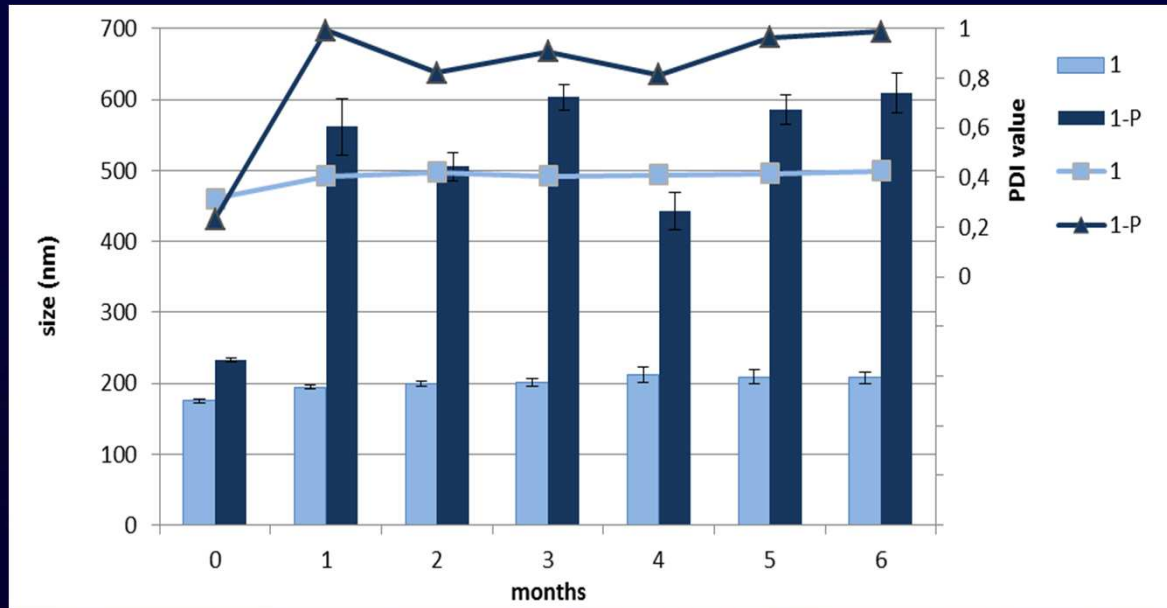
mean size: **150-300 nm**

Zeta potential up to **+60 mV**

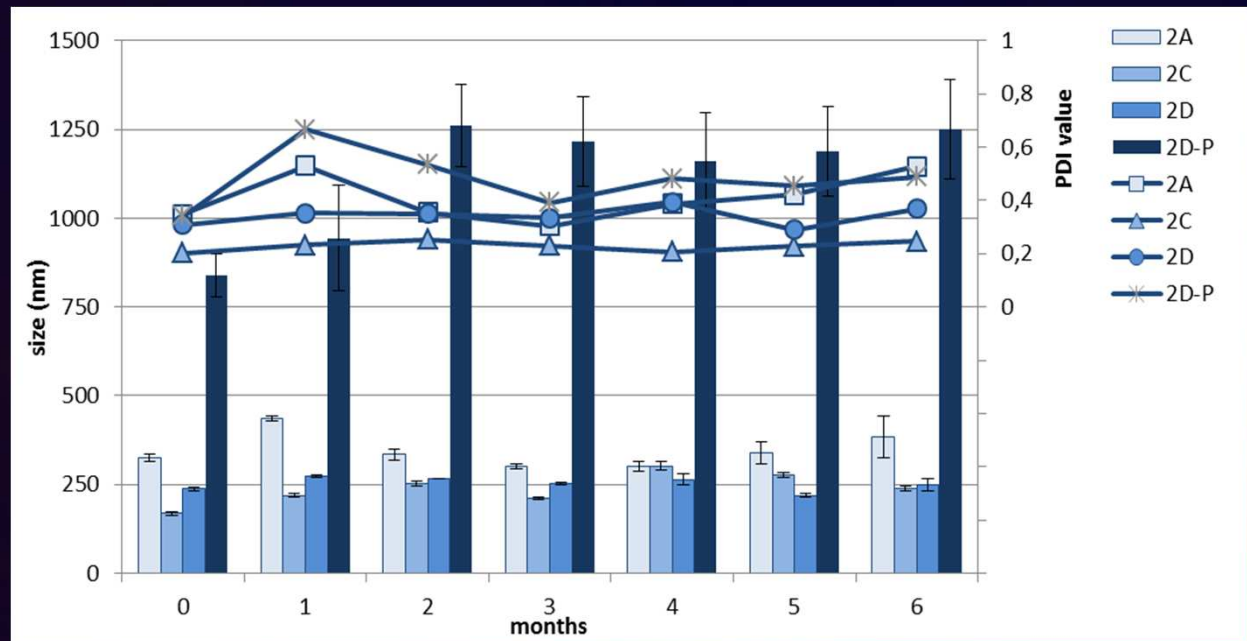
sterilizable (by autoclave or filtration)

QESD features:

- ✓ Low working temperatures
- ✓ No or low surfactant concentration
- ✓ ICH Class 3 solvents



Mean size and PDI changes upon storage of cSLN at 4 °C.



Ocular tolerability

Modified Draize test (Bucolo et al., 2004)



The tested nanocarriers did not cause ocular inflammation or tissue alteration in the rabbit eye.

Scores for conjunctival congestion, swelling and discharge were zero for all the experiments, except than congestion at 10 min (score 1).

Iris hyperemia and corneal opacity scores were also nil in all the observations.

In vivo assays



Intraocular pressure was measured in male New Zealand albino rabbits, using a Tono-Pen XL tonometer (Mentor; Norwell, MA) calibrated according to the manufacturer's instruction.

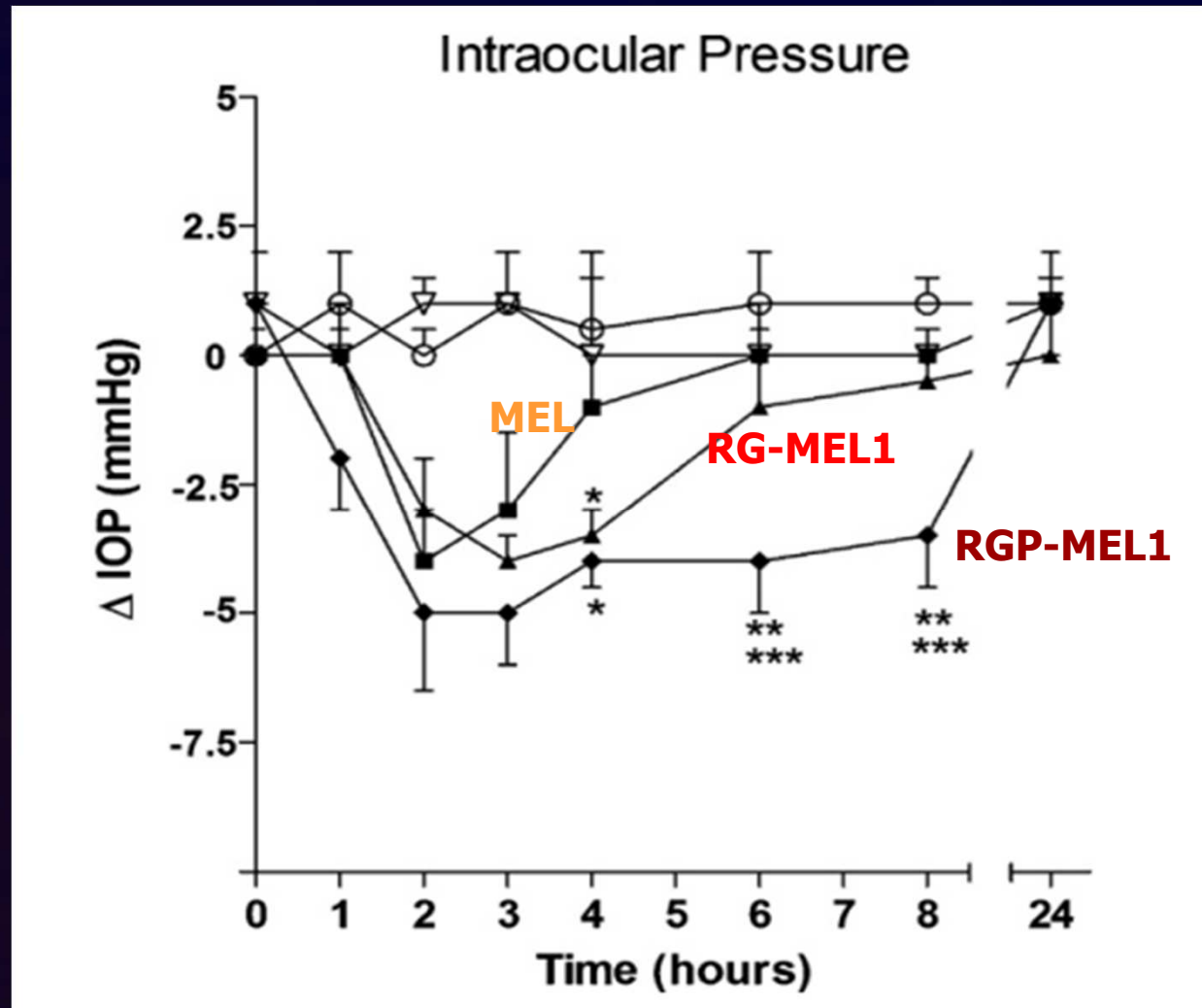
Before tonometry, 10 μL of 0.4% oxybuprocaine hydrochloride was applied to the corneas to minimize any discomfort to the animal.

Intraocular pressure in normotensive rabbit eyes after topical instillation of a MEL aqueous solution (□) or NPs: RG (○), RGP (▽), RG-MEL1 (△), RGP-MEL1 (◆).

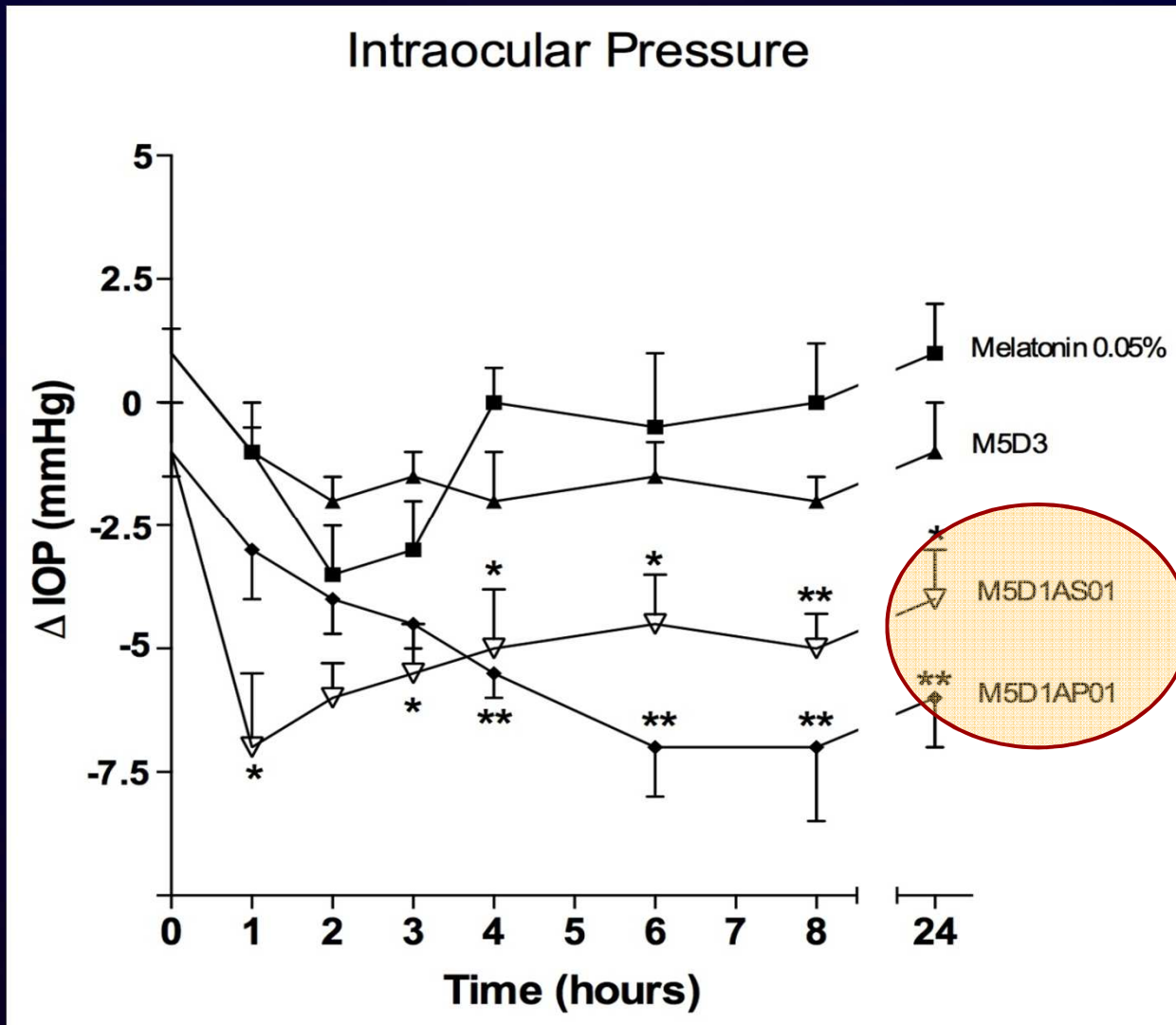
*p < 0.01, **p < 0.001 vs. MEL

***p < 0.001 vs. RGP-MEL1

**PLGA &
PLGA-PEG
NP**



IOP in normotensive rabbit eyes after topical instillation of MEL aqueous solution (□) or MEL-loaded SLN: RGP (▽), RG-MEL1 (△), RGP-MEL1 (◆).
 *p < 0.05, **p < 0.01 vs. MEL



SLN

Conclusions - I

Melatonin can be efficaciously encapsulated in polymeric or lipid nanoparticles, showing good technological properties and stability.

Conclusions - II

**Plain as well as drug-loaded
nanoparticle suspensions
showed a complete
ocular tolerability in rabbit.**

Conclusions - III

In vivo, both SLN and polymeric NPs ensured an activity comparable or higher than MEL eye-drops, but with a much longer duration of the IOP-reducing effect.

Conclusions - IV

The positive technological features (like the possibility to sterilize or freeze-dry) are interesting for a further optimization of these nanotech formulations.

Credits

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