

Evaluation of the effects of TiO_2 nanoparticles conjugated with fluorescein on retinal angiography

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BRAZIL

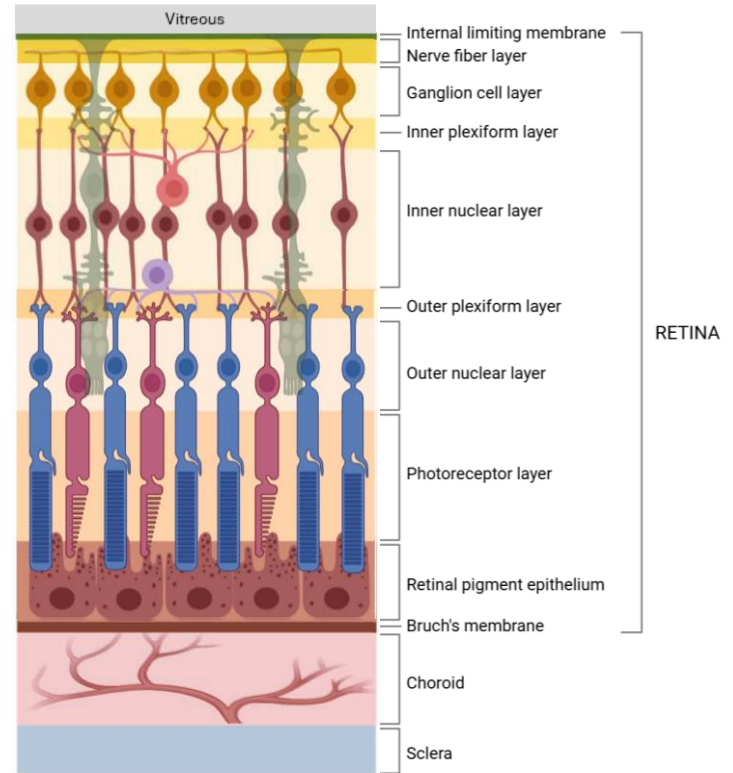
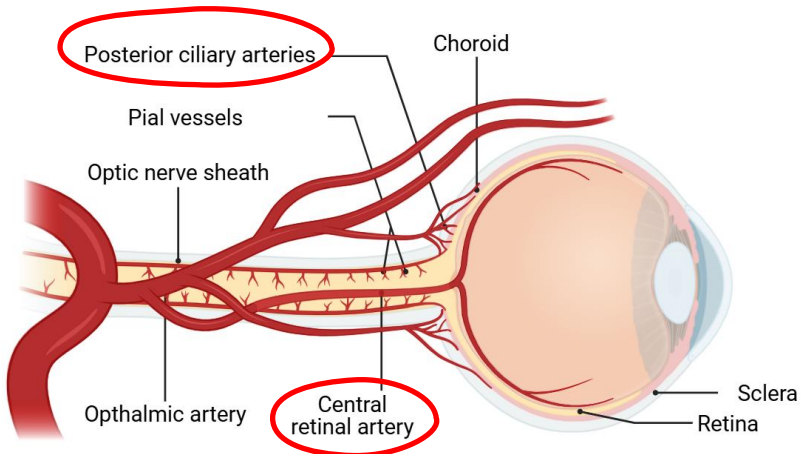


Biodegradable implants for
diseases of the posterior
segment of the eye

Nano delivery systems for
topical and intraocular
application

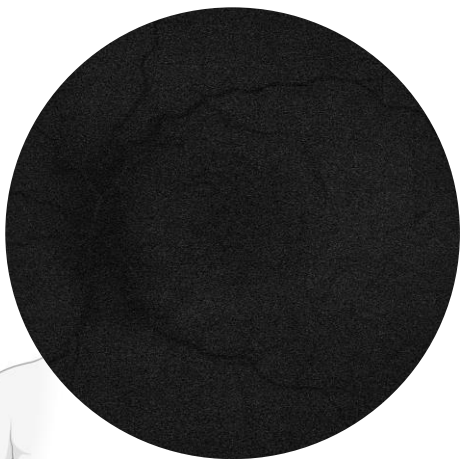
- Fundus Fluorescence Angiography
- Ultrasmall nanoparticles as vehicles for contrast agentes
- Ultrasmall nanoparticles conjugated to fluorescein

Blood supply of the retina



Fundus Fluorescence Angiography (FFA)

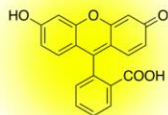
Invasive
diagnostic
procedure



Sodium fluorescein

- Low molecular weight (376.3 Da)
- Absorption: 465 to 490 nm (cobalt blue light)
- Emission: 520 to 530 nm (green light)

Adult dose = 500mg



**Microvascular
abnormalities
in retina and choroid**

neovascularization
non-perfused areas in the retina
blood vessel leakage
inflammation



DISEASES

Diabetic Retinopathy
Age-related macular degeneration
Retinopathy of prematurity
Retinal vein occlusions



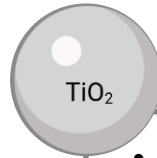
Complications

nausea
vomiting
bradycardia
hypotension
allergic reactions

Nanoparticles-based contrast agents



Improve the quality of
image-based diagnostic exams



- Chemical stability
- Affordability
- Versatility
- Low toxicity

Ultrasmall NPs (< 5nm)

Favorable PKC characteristics
Rapidly elimination

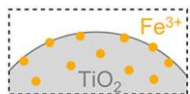


Renal clearance
Excreted by urine

.... Previous work

Ultrasmall titanium dioxide nanoparticles (NPTiO₂)

Ultrasmall NPTiO₂ + Fe (III)



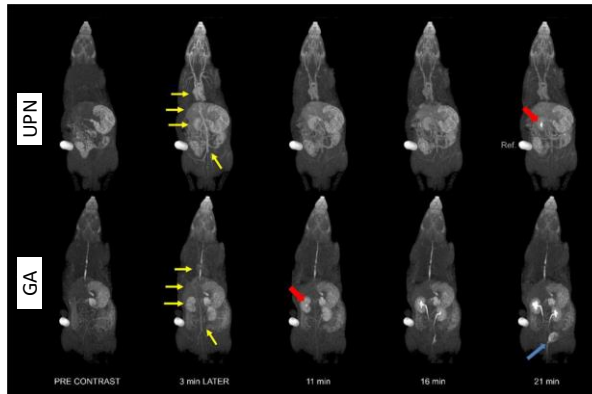
UPN
3 nm

X

Gadoteric acid (GA)

intravenous injection

Magnetic Resonance Imaging

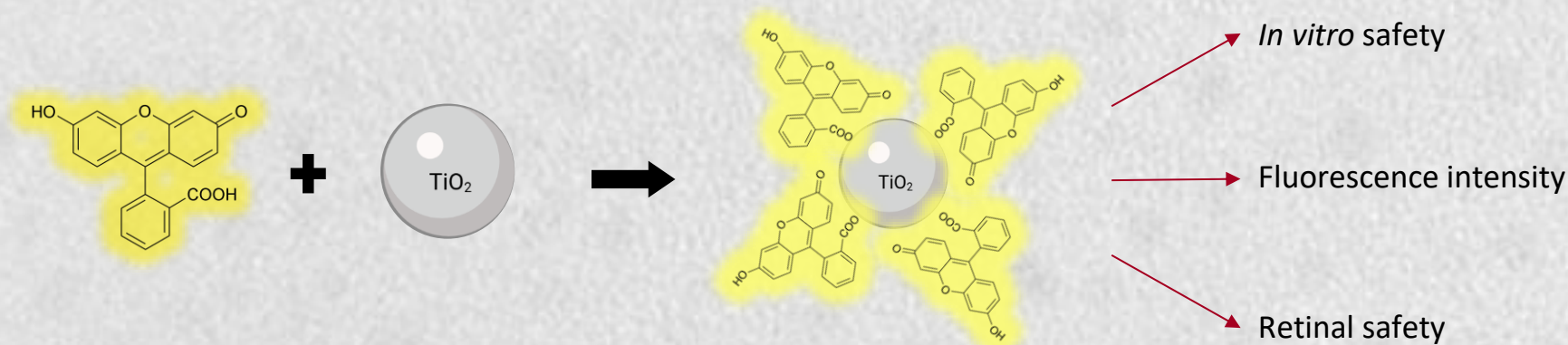


- contrast enhancement (heart, liver, kidneys, blood vessels)
- arrival and accumulation on the renal calyx
- bladder

- ✓ Preferential biodistribution in highly vascularized organs
- ✓ Renal clearance occurred only twice the time
- ✓ Completely excreted after 24hs

Nano Lett. 2023, 23, 12, 5497-5505

Ultrasmall titanium dioxide nanoparticles conjugated to fluorescein (NPTiO₂-FL)



Methods. 2025, 233, 30-41

Nanoparticles preparation

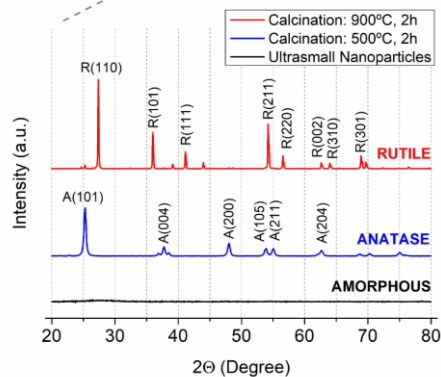
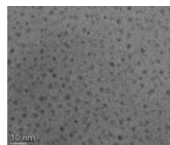
NPTiO₂

NPTiO₂ synthesis • method: thermal decomposition
• temperature: 300°C

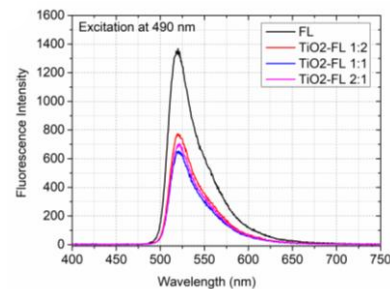
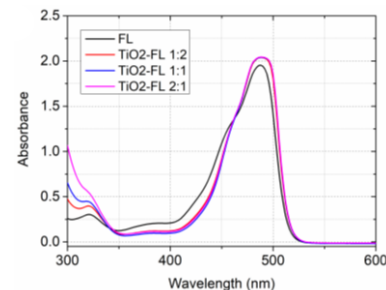
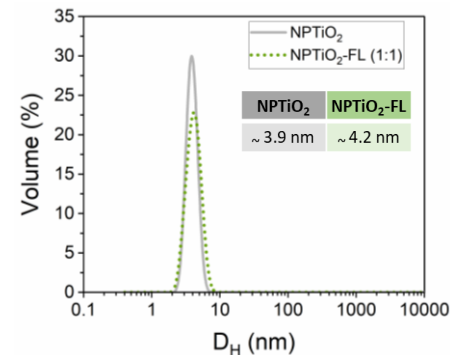
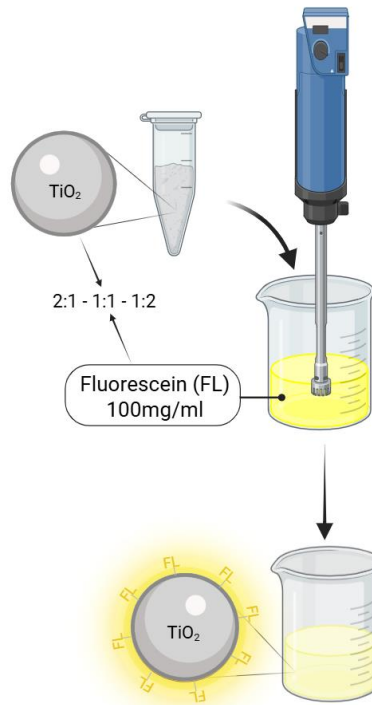
NPTiO₂ stabilization • citrate ligands

NPTiO₂ purification

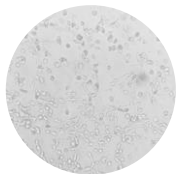
NPTiO₂ drying



NPTiO₂- FL

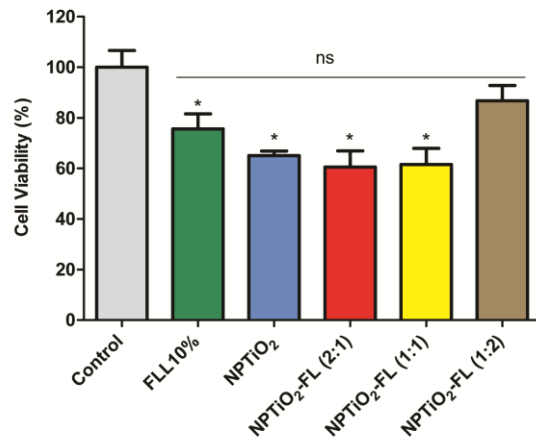


In vitro safety



MTT assay

- Huvec cell line (PCS-100-010, ATCC)
- M199 medium + 10%FBS + 1% antibiotic/antimycotic
- 3×10^4 cells/well
- 24h



NPTiO₂-FL are safe for Huvec cells

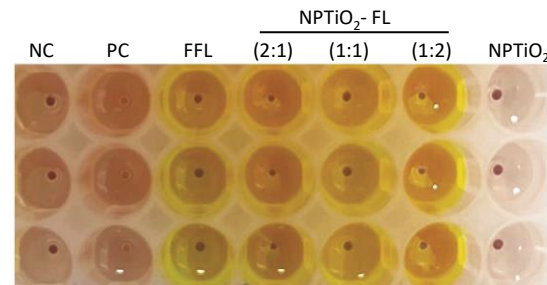


Hemolysis test

- Rats' blood
- 1% erythrocyte suspension (1:1 ratio)
- Triton (positive control); PBS (negative control)
- $\lambda = 405\text{nm}$

$$HR(\%) = \frac{OD_{test} - OD_{neg}}{OD_{pos} - OD_{neg}} \times 100$$

Odtest – optical density of the test sample
 Odneg – optical density of the negative control
 ODpos – optical density of the positive control

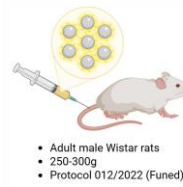


**NPTiO₂-FL
 have good
 hemocompatibility**

NPTiO₂ (30 ug/ml): HR<1%

NPTiO₂-FL and FL: erythrocyte pellet ~ negative control

Fundus Fluorescence Angiography



Experimental Groups

- G1: Free fluorescein 10 % (m/v)
- G2: Free fluorescein 5 % (m/v)
- G3: NPTIO₂
- G4: NPTIO₂-FL (2:1)
- G5: NPTIO₂-FL (1:1)
- G6: NPTIO₂-FL (1:2)

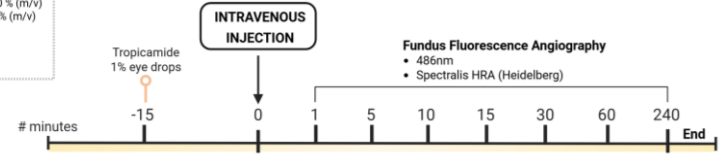


Image Processing and Analysis

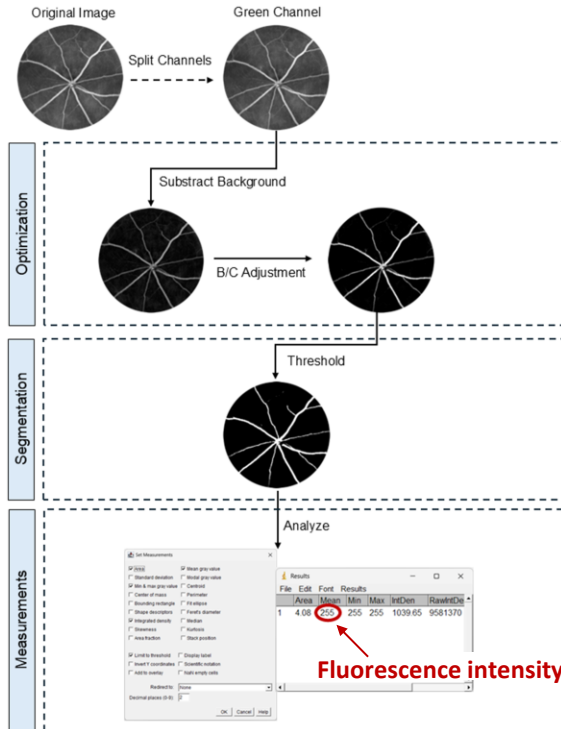
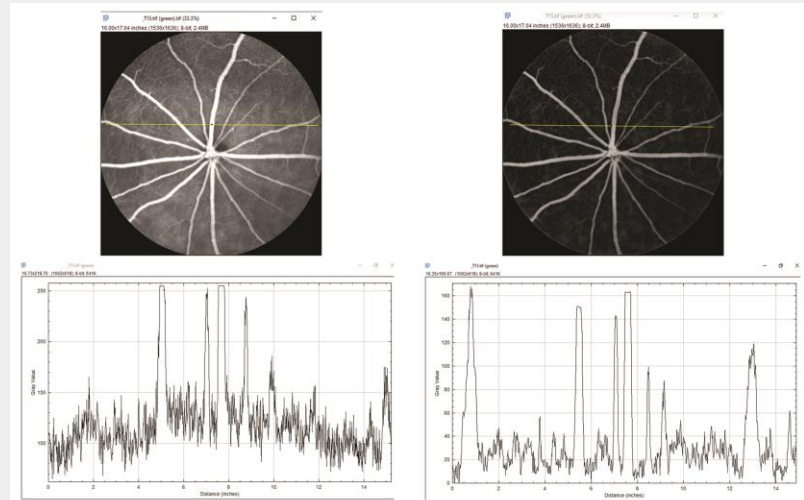


Image optimization

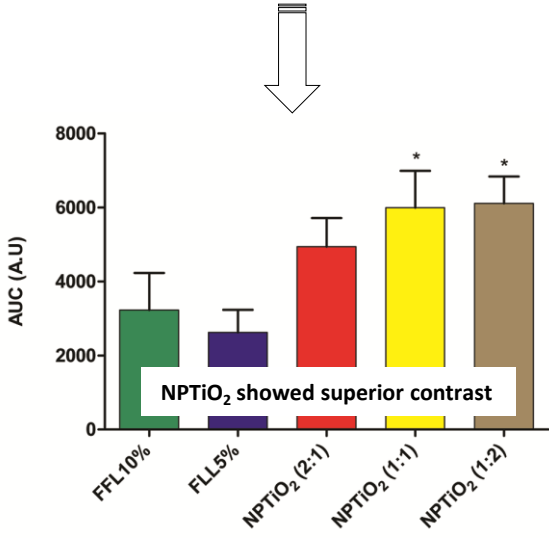
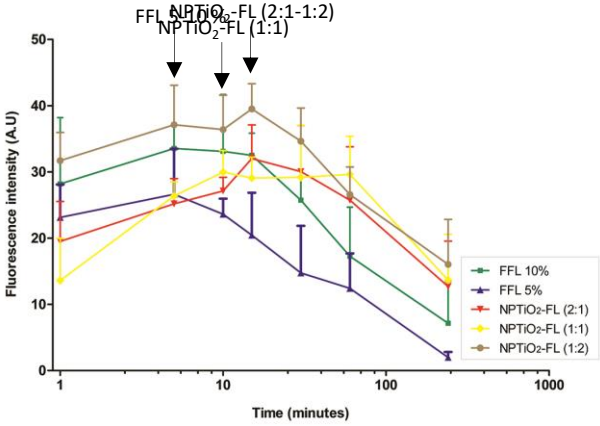
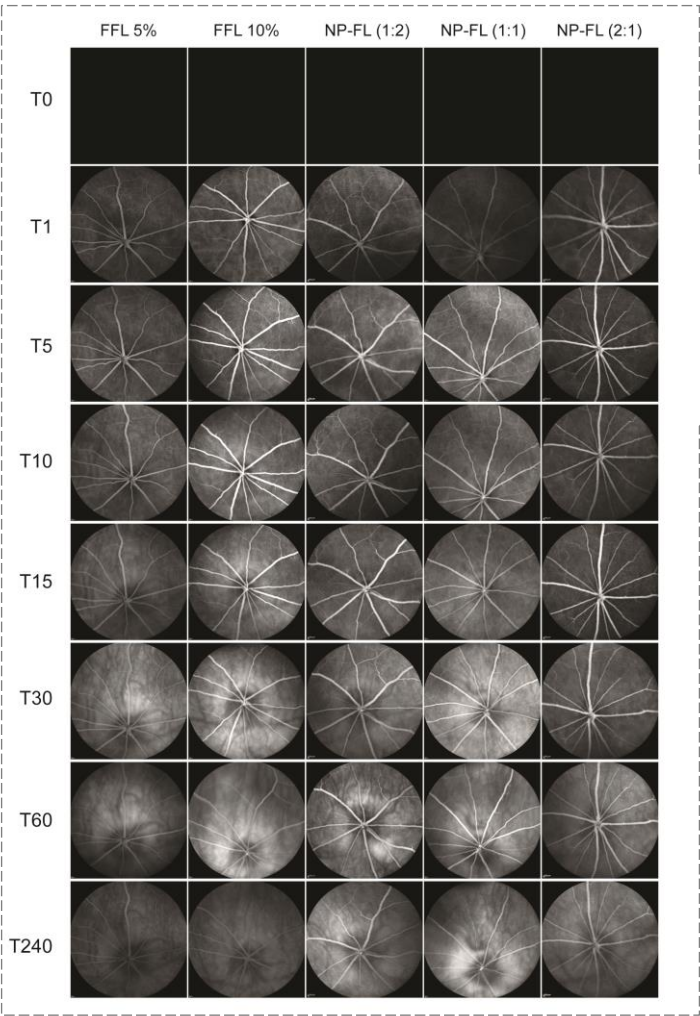
Before

After

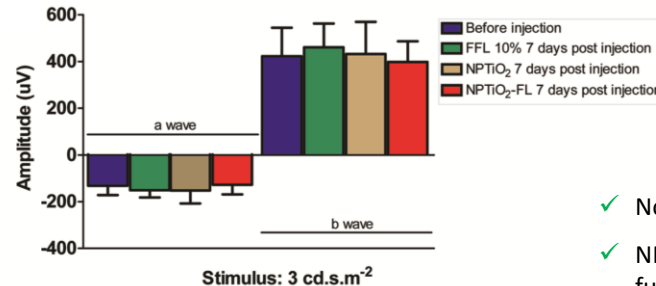
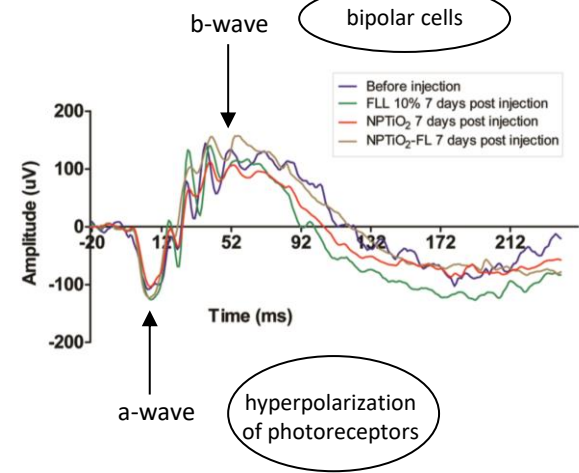
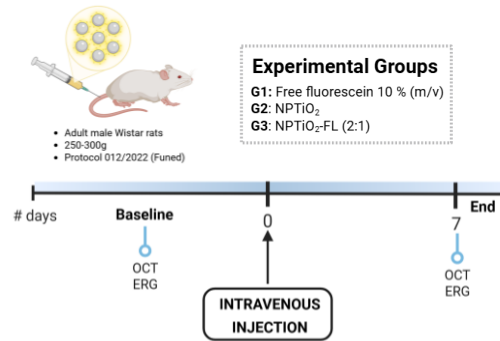


Clear distinction of smaller vessels from the background

Fundus Fluorescence Angiography analysis



Electroretinography



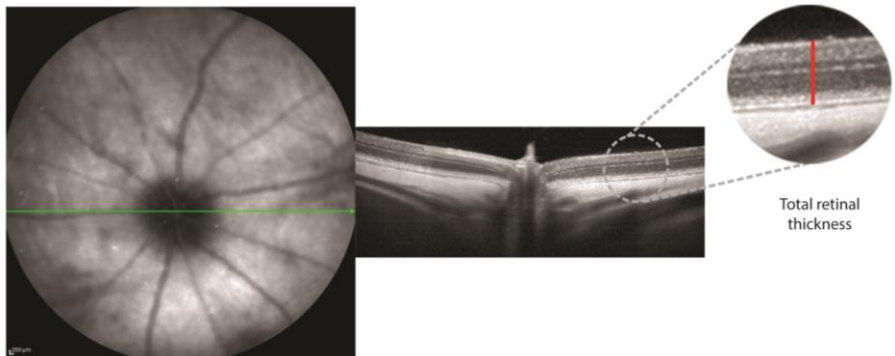
- ✓ No significant difference in a- and b- waves
- ✓ NPTiO₂ and NPTiO₂-FL did not cause retinal function damage

Optical Coherence Tomography (OCT)

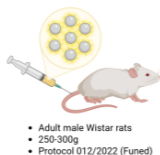


• Segmented SD-OCT images

• Heidelberg Engineering



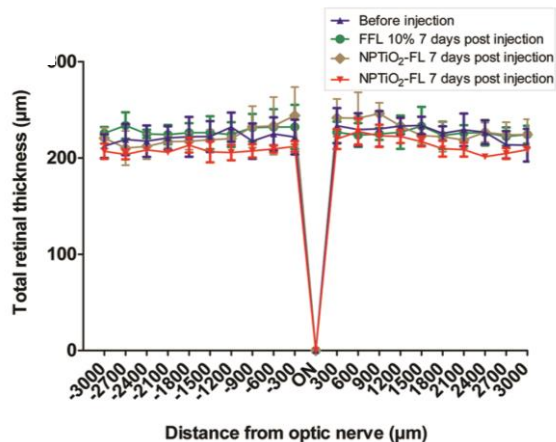
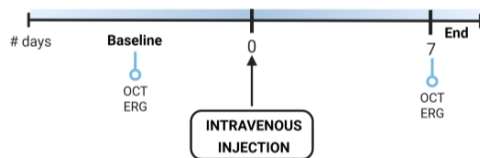
Total retinal thickness



Experimental Groups

- G1: Free fluorescein 10 % (m/v)
- G2: NPTiO₂
- G3: NPTiO₂-FL (2:1)

- Adult male Wistar rats
- 250-300g
- Protocol 012/2022 (Funded)



✓ No change in retinal thickness



No edema or inflammation

CONCLUSIONS

- ✓ **Fluorescein was successfully conjugated to NPTiO₂**
- ✓ **Enhanced fluorescence photostability:** potential to provide more detailed and informative images in angiography studies even with the reduction of fluorescence intensity
- ✓ **Enhanced retention time in the retina:** increase blood circulation time and contact with the blood-retinal barrier (BRB) compared to free fluorescein
- ✓ **Completely eliminated after 24hs**
- ✓ **Safe:** not supposed to cause hemolysis and do not change retinal thickness and function

Acknowledgments



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- Dr. Marina F. Dias
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**NEXT-GENERATION
DELIVERY INNOVATIONS**

Thank You!

For Connection!



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