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EVALUATE NANOPARTICLES AS VEHICLE FOR SIR RETINAL DELIVERY

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Abstract

Treatment of many retinal problems today requires invasive intravitreal injections of highly concentrated medications. Because of the possibility for significant complications after monthly injections, such as infections, hemorrhage inside the eye, or retinal detachment, many doctors recommend reducing the injection frequency and the desired medication dosage rather than increasing it. As a result, it's preferable to have a release that lasts for a while in the area of interest. In this research, we investigated the NPs' potential use as a medication carrier to the retina by analyzing their distribution and tropism after intravitreal injection.

Keywords: Retina; drug-delivery; lipid-DNA nanoparticles; fluorophotometry; biodegradable;

INTRODUCTION

Medication is injected into the eye to treat various retinal disorders. The intravitreal route is the most common, however the periocular route is sometimes employed. There are several difficulties in getting drugs to the retina. The most obvious problem is finding the right approach to applying it. The blood-retinal-barrier prevents drugs from entering the eye from the circulation, making local administration preferable to systemic exposure. Nevertheless, owing to tissue features and clearance processes, Drugs administered topically have a hard time getting from the anterior chamber of the eye and through the vitreous to the back of the eye, where the retina is located. Subretinal injections are the most relevant use nearby, but they carry the dangers associated with surgery and can only affect the little portion of retina right next to the injection site. As a result, intravitreal injections constitute the norm rather than the exception.

Several novel medications with unique formulations have been created to target delivery through changed techniques and routes, in the hope of enhancing the efficiency of medication delivery to the back of the eye with minimal side effects. To do this, it is possible to use eye drops or other minimally invasive injectable procedures to slow the rate at which the medicine enters the posterior ocular region. Biodegradable, biocompatible, non-antigenic, inexpensive, and easily accessible, gelatin stands out among them as an FDA-approved safe product.

The goal of this study was to ascertain whether the concentration of HA on the surface of biopolymeric nanoparticles affected their efficacy in transporting drugs to the retina. (Figure 1a). The effectiveness of eye drops and subconjunctival injection in delivering GEH in +/ surface charge was investigated in a mouse model (see Figure 1b). Cryosections of the whole eyeballs with fluorescence were analyzed quantitatively to track eye tissue movement.

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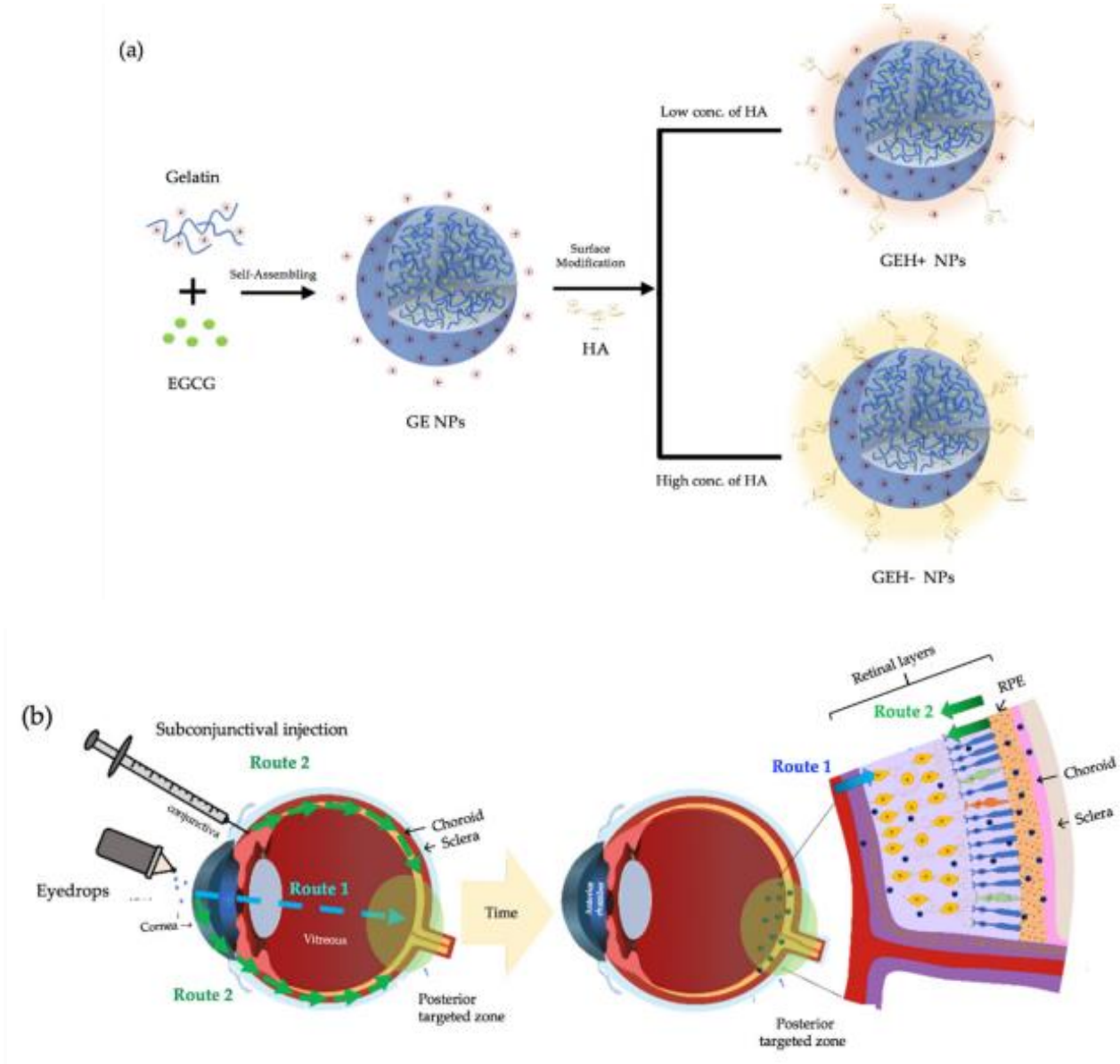

Figure 1: Schematic drawing

LITERATURE REVIEW

Chien, Y., Hsiao, YJ., Chou, SJ. *et al.* (2018), One of the most common causes of blindness in the world is inherited retinal diseases (IRDs). Due to their complexity and genetic variability, however, the vast majority of these conditions have no reliable therapy option. Inherited regressive diseases (IRDs) that were formerly considered to be fatal may now be treatable using gene therapy. For gene editing to be effective, however, CRISPR-Cas9 components must be efficiently delivered into the intricate 3D structure of human retinal tissue. Recent promising research in the area of nanoparticles (NPs) has made significant progress toward therapeutic uses of CRISPR-Cas9. The use of induced pluripotent stem cells (iPSCs) and 3D retinal organoids cultured in vitro has also cleared the road for future CRISPR-Cas9 clinical studies for the treatment of IRDs. In this review, we will look at the progress that has been made in the treatment of IRDs using NP-based gene therapy, the CRISPR-Cas9 system, and retinal organoids produced from iPSCs.

Jiao, Zhang, Niu, Gao, Zhang, Yu, Yang, and Liu. In 2015, several medicines have limited bioavailability because of the complicated drug delivery barrier in the eye. Testing novel medications

through suitable transport mechanisms is essential. Possible ocular drug delivery strategies including interaction with the ocular mucosa, drug retention for a longer period of time in the eye, and increased permeability have been developed via the use of different nano-carriers thanks to advances in nanotechnology. In addition, More and more people are turning to theranostic applications, which use nano-carriers including liposomes, nanoparticles, nanosuspensions, nano-micelles, and nano-emulsions to combat resistant microorganisms.

Yuhong Wang & Ammaji Rajala, 2018, There has been significant development in our knowledge of the molecular pathways underlying the pathophysiology of retinal degenerative disorders in recent years. This insight has prompted the study of gene therapy methods for curing these fatal diseases. A potential therapy for retinal degenerative illnesses is non-viral gene transfer. In this study, we will briefly discuss the composition, properties, and current use of three different nanoparticles (NPs) for ocular treatment.

Srinivas, S.P.; Kaphle, A.; Navya, P.N. The limitations of current medicinal formulations may be overcome by using nanotechnology, as suggested by et al. (2019). Much progress has been made toward the use of engineered nanomaterials for the highly precise, sensitive, and effective therapy of cancer. In order to successfully target cancer cells and deliver encapsulated payloads, tailor-made nanoparticles functionalized with appropriate ligands are required. Nanotechnology, according to the available literature, will offer cutting-edge platforms for cancer care and anticancer treatment. Hence, in this critical review, we explore the basic importance of the physicochemical features of a variety of nanomaterials that are now being used for anticancer therapy.

Mazayen, Z.M., Ghoneim, A.M., Elbatanony, R.S. *et al.* (2019), The synthesis method and the physicochemical properties of the raw materials used to create the nanoparticles have a significant impact on the final product. Several different nanodrugs are now being tested in clinical trials, and many of them have already found utility in both diagnostic and therapeutic settings. Nanoparticles have found use in the treatment of renal illness, TB, skin disease, Alzheimer's disease, various cancers, and the development of the COVID-19 vaccine.

Materials and Methods

We bought all of our chemicals and reagents from commercial sources and utilized them as is. The University Pharmacy at Tübingen supplied the mydriatic agent, which included Phenylephrin-HCL (225 mg), Tropicamid (45 mg), Povidon 25 (270 mg), and Aqua ad. inj. (8.36 g) in a total volume of 9 milliliters (mL).

Preparation of Buffers and NPs

This yielded Tris-buffered saline (TBS). To bring the concentration of the 10 solution down to 1 L, ultrapure water was added. Before using, we diluted the sample by a factor of ten using sterile water. The addition of acetic acid increased the pH to 8.0. The last step was to fill it to 1 L with distilled water. In an Eppendorf tube, 200 liters of water, 5 millimolar sodium chloride (MgCl2), and 100 liters of 50 TAE stock were combined to form NP stock buffer. The optimal concentration of NP buffer.

Finally, the NPs and hybridization were produced with the use of a temperature gradient. The thermocycler was used to heat the NP solutions to 90 C and keep the temperature stable for 30 minutes. Variations in DNA length and lipid residue count were tested using various DNA monomers. The defining features of each NP are summarized in Table 1. The features of the oligonucleotide are hinted at in the name of the corresponding NP. The first capital letter "U" denotes that a lipid-modified nucleotide was used. The number that comes after the U represents the total number of deoxyuridine nucleotides that have been chemically altered. The moniker "T" oligonucleotide indicates that the

altered nucleotides begin at the 50' end of the molecule.

Table 1: Sequence and details of lipid-modified oligonucleotides used for NP preparation.

Name	Sequence (5′->3′)	Lipid Modified Bases # (%)
U4T-12	UUUUGCGGATTC	4 (33)
U4T-18	UUUUGCGGATTCGTCTGC	4 (22)
U6T-12	UUUUUUGGATTC	6 (50)
U6T-18	UUUUUUGCGGATTCGTCT	6 (33)
U6T-20	UUUUUUGCGGATTCGTCTGC	6 (30)

Fluorophotometric Measurements

Vitreous fluorescence was measured using an ocular fluorophotometer, as has been previously described. Before taking any readings with the Fluorotron MasterTM, the target eye was centered in the system's lens and on the optical axis. A software supplied by Ocumetrics, Inc. recorded the fluorescence in 0.25 mm sections along the specified optical axis, and then converted the measurement to the appropriate concentration in ng/mL. At each step point, the Fluorotron counts photons and uses the following formula to calculate the fluorescein concentration in terms of ng/mL.

Fluorophotometric Measurements

The NPs were seen using fluorescence microscopy thanks to the covalent attachment of the fluorescent dye Atto488 to their complementary strand. The fluorescently tagged NPs were created by hybridizing this strand with the NPs. The chemical structure of DNA is changed when this dye attaches to it and, as a consequence, its fluorescence intensity. The associated DNA sequence determines the amount to which such changes take place. Due to the fact that each NP is made up of a little different sequence (in terms of both base length and the degree of lipid modification), the dye takes on somewhat different intensities for each NP. Because of this, it is impossible to compare the fluorescence intensity of one NP to that of another NP or to that of the free dye. Each NP had its own unique baseline value set at 100% so that the findings discovered for them could be compared. All subsequent measurements of that NP structure were normalized to this value for ease of comparison to other NPs.

Normalization was performed using two distinct initial values. Before injecting the sample into the fluorophotometer, we first normalized the data by measuring the concentration of each NP in solution inside a cuvette. This number indicates what percentage of the injected quantity has been identified. From here on, it shall be referred to as the "solution" measurement. The second was taken immediately upon injection and is shown as the "instant" time point in the subsequent figures. This number provides light on how the NPs circulate across the vitreous body over time.

Intravitreal Injection of NPs into Ex Vivo Pig Eyes

Fresh porcine eyeballs were purchased from a nearby slaughterhouse, stored at 4 degrees Celsius, and processed within 4 hours of arrival. The eyes were quickly rinsed with phosphate-buffered saline (PBS) and allowed to warm up to room temperature before receiving NPs or control. While the NP was being injected, one hand was used to keep the eyeballs in place on the bottom of a petri dish. Then, a 30-gauge needle was used to penetrate the pars plana 4 mm beyond the limbus of the eye. After being exposed to PBS for the specified amounts of time, the pigs' eyes were analyzed.

Before injection, at 0, 5, 15, and 30 minutes, and after 4, 8, and 24 hours of incubation (n = 6-8), fluorophotometric measurements were taken. After 5, 15, 30, 1, or 2 hours after incubation, the pig eyeballs (n = 3) were frozen in liquid nitrogen and implanted in Tissue-Tek for microscopic examination. The next step was to prepare, stain, and photograph cryosections.

Intravitreal Injection of NPs into In Vivo Rat Eyes

A 0.1 mL/kg bodyweight solution of a three-component anaesthetic was administered into the peritoneum of the animals to put them to sleep for the surgery. Fentanyl (0.005 mg/kg), Midazolam (2.0 mg/kg), Medetomidin (0.15 mg/kg), and 0.9% NaCl (in a total volume of 0.5 mL) were used to create this anesthetic. Fluorophotometric readings were taken before injection and again at 0, 1, and 5 days thereafter. To do this, eyes (n = 6) of awake rats were dilated with the mydriatic agent before they were anesthetized in the manner described above. The antidote was injected, and the anesthesia wore off.

Injection of NPs into Periocular Tissue of In Vivo Rat Eyes

Subconjunctival injection of 20 mM NP solution (40 L) into the periocular region was conducted. Using a microscope, an incision was made in the conjunctiva about 10 mm temporolateral from the most lateral point of the limbus, at a depth of about 5 mm. After the cataracts were taken out, the affected eyes and the subconjunctival tissue around them were snap frozen in Tissue-Tec.

Fluorescent Microscopy and Imaging

Tissue-Tek O.C.T. embedded porcine and rat eyeballs were frozen, sectioned longitudinally using a cryostat, thawed, mounted on glass slides, and maintained at 30 C until use. Before being washed three times for three minutes in 1 TBS solution, each slice was fixed in 300 L of methanol for 10 minutes. After five minutes, the nuclei were stained with 100 L of 0.4 g/mL 40,6-Diamidino-2-phenylindol (DAPI), and the cells were washed three times for three minutes in 1 TBS buffer. When the dye on the slides dried, we covered them with FluorSave, and we also supplied cover glasses. After then, fluorescence microscope images of the samples were taken. Images of the nuclear stain (DAPI) and the NP-conjugated fluorescent dye (FITC) were taken using a DAPI and FITC small band filter, respectively.

Results

To determine if DNA NPs might be useful drug-delivery vehicles for the treatment of retinal diseases,

their distribution and retention duration were examined ex vivo and, subsequently, in vivo. Intravitreal injection was utilized on porcine eyes removed from living animals. The top two performing NPs were selected for further in vivo testing. Both intravitreal and subconjunctival injections were tested in vivo to determine which was more effective.

Intravitreally injected NPs were used to study their uptake and distribution in ex vivo pig eyes. Ocular fluorophotometry was used to track the NPs' fluorescence for up to 24 hours. This apparatus utilizes a series of focal spots located along the optical axis within the ocular cavity to calculate intensity as a function of depth, as described in Section. If these signals are integrated, a fluorescence profile may be generated that is directly related to the amount of NPs used (Figure 2). The first reading was obtained soon after the NP injection. At 5, 15, 30, and 1 hours, the signal was evaluated again. Immediately after NP injection, the vitreous body had the greatest signal (Instant). About 270 units of fluorescence intensity was measured here. Over time, the signal faded, suggesting that the NPs were leaving the eye through diffusion. (Figure 2).

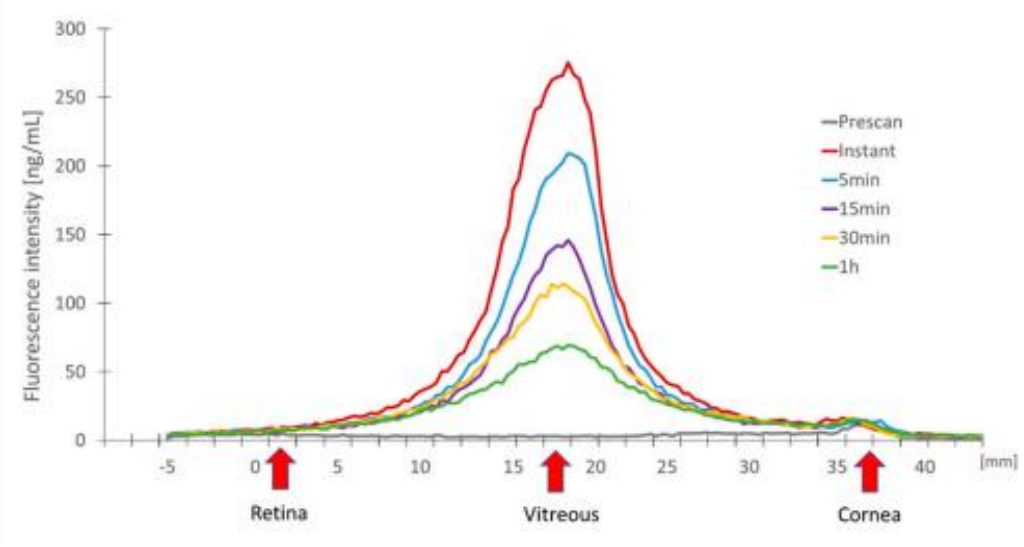

Figure 2: Intravitreal injection of U4T-18 NPs along the optical axis (x) in ex vivo porcine eyes, with fluorescence intensity (y) evaluated at various times.

To calculate the NP concentration in each eye, we integrated and normalized the fluorescence intensity in the vitreous body during a fixed time interval, as described in Section. Basic molecular control of the NPs was carried out using free Atto-488. To facilitate comparisons across different NPs, both the fluorescence intensity of the solution before to injection and the first measurement taken post-injection were used to normalize the results. Natural fluorescence of the pig eyes used was also plotted so that results could be compared to those obtained before and after injection.

Twenty-four hours after the injection, fluorescent readings were collected. The data were first normalized with respect to the "solution" value (Figure 3A). The detected percentage of the total injected quantity may be determined by analysis of the NPs normalized to their respective solution value. Another normalization method was used, which focused more on the NPs' spread and survival after injection. The second approach used NP-specific fluorescence and instantaneous measurement normalization to accomplish this (Figure 3B).

U4T-12 showed superior retention qualities over time compared to the first fluorescence following injection (Figure 3B). Only U4T-12, out of all the NPs tested, showed an increase in fluorescence intensity beyond the immediate value; this rise peaked 30 minutes after injection. U4T-12 fluorescence intensity remained greater than the immediate value for at least two hours after injection, gradually

declining afterwards. Immediately after injection, levels for all other NPs dropped below those of the control group. Five minutes (78.0%) and fifteen minutes (63.80%) after injection, only U4T18 showed values lower than the control. After 8 hours, the fluorescent intensity of U4T-12 is 41.6% and that of the other NPs is 22.6% of the immediate value, while the control is below 10%.

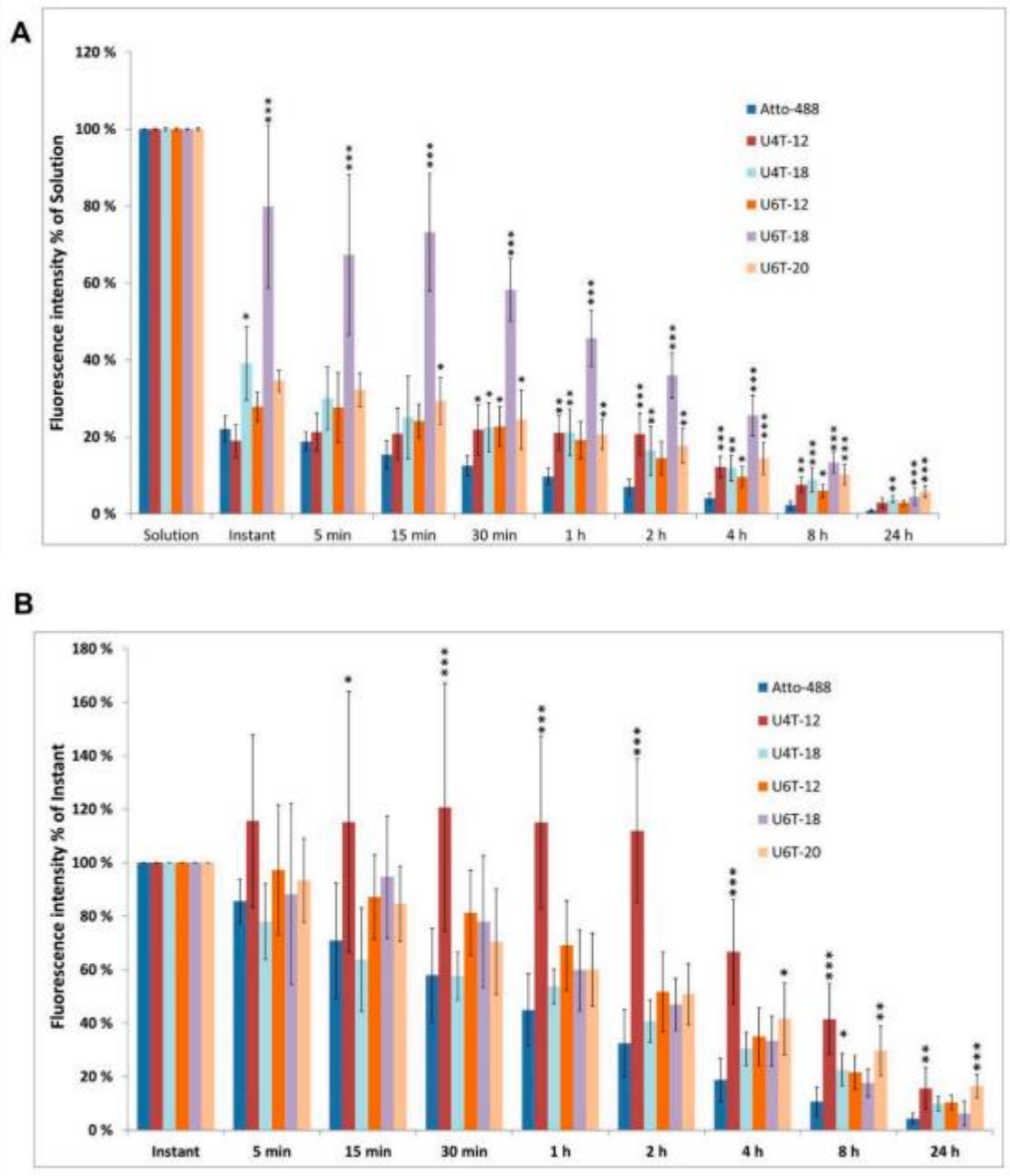

Figure 3: Fluorescence intensity of different NPs normalized to the "solution" value.

CONCLUSION

Although the small molecule control rapidly diffused away, the various assessed NPs remained concentrated in the vitreous body and retina of ex vivo pig eyes for 24 hours. While NPs diffuse slowly, a 5-minute incubation time reveals their presence at the retina, suggesting robust interactions with this tissue. These results are consistent with those shown in the ex vivo system. Finally, we found that our NPs bind to the retina both ex vivo and in vivo over a period of days. These encouraging findings encourage additional investigation into creating lipid-DNA NPs as a drug delivery platform for the

posterior segment and testing the effectiveness of the delivered medicine.

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