



Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: [www.sciencedirect.com](http://www.sciencedirect.com)

Original article

# Promise of latanoprost and timolol loaded combinatorial nanosheet for therapeutic applications in glaucoma

Li Wang<sup>a,\*</sup>, Yan-Yun Jiang<sup>a</sup>, Nan Lin<sup>a</sup><sup>a</sup> Department of Ophthalmology, Tongren Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200336, China

## ARTICLE INFO

### Article history:

Received 8 September 2019

Revised 23 September 2019

Accepted 26 September 2019

Available online 11 October 2019

### Keywords:

Glaucoma  
Nanosheet  
Latanoprost  
Timolol  
Cornea  
Ocular

## ABSTRACT

To demonstrate a combinatorial drug loaded nanosheet made of alginate and chitosan which reduces intraocular pressure in a slow and sustained manner depicted by in-vivo studies. The combinatorial drugs loaded were latanoprost along with timolol and nanosheet was termed LATINA. LATINA was fabricated using spin coating procedure and characterized using scanning electron microscopy, atomic force microscopy, adhesive peel strength, burst and cytotoxicity tests. In-vitro studies of drugs were also performed. It was clearly seen that LATINA was stable, flexible, durable, biocompatible and biodegradable drug delivery system. The main advantage provided by the nanosheet technology is the properties which could be controlled by altering the thickness of the sheet. The slow and sustained drug release from LATINA ensured that the nanosheet may be renewed once weekly instead of daily as for other ophthalmic formulations for glaucoma. This also led to reduction of intra ocular pressure (IOP) of eye. LATINA was also found to be non-irritable to the cornea of eye.

Hence, it may be safely concluded that with additional research, LATINA may prove to be the best option for drug delivery to the cornea.

© 2019 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Clinical biotherapeutics and diagnostics have attained new standards with the advancements in bioengineering. Nanobioengineering generates tailor-made novel sensors and drug delivery systems which cater to individual maladies (Weng et al., 2017). The novel efforts are enormous in cases of development of smart nanobiomaterials which may minimize the risk of side effects like development of new infections (Atabay et al., 2017). A class of these smart nanobiomaterials are nanosheets which are ultra-thin sheets of polymer which have remarkable nanothickness and unique properties which may be controlled in a manner that may be thickness dependent (Forrest and Dalnoki-Veress, 2001; Okamura et al., 2015; Ricotti et al., 2010). Nanosheets are also

highly flexible and biodegradable, hence with any anti-glaucomic drug may prove to be an ideal drug delivery system for reduction of glaucoma (Fujie et al., 2007; Okamura et al., 2015; Kim et al., 2014). Glaucoma is an ophthalmologic condition which may impair vision irreversibly (Nair et al., 2016; Natarajan et al., 2014) and requires extreme lifelong care for the person. Until now, reduction in intraocular pressure (IOP) is only the demonstrated remedy. It has been also seen that patients with glaucoma use anti-glaucomic solutions throughout their lifetime to keep the condition in check (Centinel and Montemagno, 2016). But the flip-side of this therapy is poor adherence of these anti-glaucomic solutions with decrease in adherence on increased use (Sleath et al., 2015). This topical administration may also be associated with decreased continuity (Vanelli et al., 2009; Stone et al., 2009; Bachu et al., 2018). Latanoprost is an analogue of prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) where the last three carbon atoms on the omega chain have been replaced by a phenyl group (Alm et al., 1995; Hotehama et al., 1993; Stjernschantz, 1992; Alm et al., 1993). Latanoprost produces ocular hypotensive effect and thereby has been seen to cause IOP reduction during glaucoma (Wong et al., 2014). Timolol, on the other hand is a beta blocker which is also effective in reduction of IOP (Camras, 1996; Dubey et al., 2015). However, it has been clearly demonstrated by several studies that latanoprost is the best which lowers the IOP, is very well tolerated systemically

\* Corresponding author at: Department of Ophthalmology, Tongren Hospital, Shanghai Jiao Tong University School of Medicine, XianXia Road, Shanghai 200336, China.

E-mail address: [AmiePittsvjv@yahoo.com](mailto:AmiePittsvjv@yahoo.com) (L. Wang).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

and has a very favourable adverse-effect profile (Wentz et al., 2014; Natarajan et al., 2012). We hypothesized that loading timolol and latanoprost on the nanosheet would cause the IOP to decrease further, and would be effective for a period of time without having to renew drugs daily. Moreover, it was also hypothesized the synergistic effect of latanoprost and timolol delivered via a biodegradable nanosheet would probably be a best solution to glaucoma therapeutics. The nanosheets loaded with the drugs were prepared, characterized with the cytotoxic effects being noted and finally their performance evaluated via in-vivo studies.

## 2. Materials and methods

### 2.1. Materials

Chitosan (molecular weight 88,000) along with sodium alginate (106,000) were acquired from Mokpo (Korea). Polyvinyl alcohol (PVA) was obtained from Sigma, China. The polymers were utilized as purchased. All the other chemicals were purchased from Sigma, China.

### 2.2. Fabrication of the nanosheet

The latanoprost-timolol nanosheet (LATINA) fabrication was carried out in a clean room of class 10,000 to evade contagions of any kind. By utilizing deionized water (DI), chitosan (1 mg/mL, 1% [vol/vol] acetic acid) and sodium alginate (1 mg/mL) mixtures were made as per the previous reports (Cho et al., 2001; Jiang et al., 2004).

### 2.3. Atomic force microscopy of the LATINA

The morphology of the LATINA made was also characterized using atomic force microscopy (AFM). The thickness of the LATINA was also calculated by atomic force microscopy. The AFM measurements were executed in air by means of the tapping mode. A silicon probe was utilized with an oscillating frequency of 200 kHz for the tapping mode. 3–10  $\mu\text{m}^2$  area was scanned through 48 N/m as the force constant and the tip height was 12  $\mu\text{m}$ . Ip<sub>2.1</sub> software was utilized to analyze the images. The procedure of AFM measurements was adapted from Das et al. (2011) with proper modifications.

### 2.4. Adhesive strength of the LATINA

A micro-scratch test was done to estimate the macroscopic adhesive properties of nano-thin LATINA. The procedure was adapted with modifications from Komachi et al. (2017).

### 2.5. Burst test of the LATINA

The pressure to burst the LATINA with three to ten layers was assessed by means of a bursting test procedure established by (Fujie et al., 2009; Okamura et al., 2009; Ariga et al., 2016).

### 2.6. in-vitro release studies of LATINA

The release of latanoprost/timolol, latanoprost and timolol from LATINA was studied in vitro. The procedure was adapted with modifications from Kashiwagi et al. (2013).

### 2.7. Cytotoxicity of the LATINA

#### 2.7.1. Cell culture

Human ocular epithelial cell line was obtained from Beijing, China. For the cell culture, prearranged conditions of 37° with 5% CO<sub>2</sub> in a humid environment were created (Gong et al., 2017; Dou et al., 2017).

#### 2.7.2. Cytotoxicity test

Cytotoxicity of control and LATINAs 1, 2, 3 were evaluated according to Dou et al. (2017) with applicable changes. Following the procedure mentioned above, confluent cell cultures were obtained.

#### 2.7.3. Animals

The procedure followed was modified from Kashiwagi et al. (2013).

#### 2.7.4. IOP reduction by LATINA

IOP was measured immediately before applying control, LATINAs 1, 2, 3, latanoprost and timolol to the surface of the cornea. Subsequently, on 1, 2, 4, 7, and 9 days after application of LATINA under general anaesthesia as mentioned, IOP was measured with a rebound tonometer (TonoLab, Finland).

2.7.4.1. Estimation of eye scratching activity after application of LATINA. For this study, only 4 adult Sprague-Dawley rats were used. The designing of the experiment was subjective to the study by Kashiwagi et al. (2013).

### 2.8. Statistical analysis

Means  $\pm$  SD is the standard form of expression of all data. Statistical study was made with one-way ANOVA for various assessments. Student's *t*-test was done for evaluation among two groups.  $P < 0.5$  was deliberated to be statistically significant.

## 3. Results and discussion

### 3.1. Fabrication of the LATINA

The main advantage of fabrication of the LATINA is its biocompatibility and biodegradability which makes it an attractive option for glaucoma therapeutics. Since LATINA is prepared of biocompatible polysaccharides, alginate and chitosan, these are degraded within the eye without the trouble of removing it. Chitosan is a cationic polymer whereas alginate is an anionic polymer. This multilayered LATINA composed of anionic and cationic polymers may embed the drug(s) without the need for any modifications in chemistry or any chemical reagents. Moreover, another important benefit lies in their adhesive which may be controlled with the thickness of the nanosheet. This is to note that although nanosheets may be handled as a single nanofilm, they are actually not so. There are multiple layers within the interior spaces of the nanosheet. In our study, we had prepared a three layered LATINA along with some ten layered LATINA in order to compare the adhesiveness. The process of creating a multilayered nanosheet was difficult. We focussed on creating an alginate/chitosan multilayer over the SiO<sub>2</sub> substrate. However, the chitosan and alginate were repelled from the hydrophobic SiO<sub>2</sub> substrate. Hence, at the same time 10% PVA was being added drop by drop which improved the spin coating procedure. Therefore, the small amount of PVA is an important constituent in the formation of multilayered nanosheet. Another delicate mechanism is the removal of the LATINA from the substrate. There was a vulnerability that the mul-

tilayers of the LATINA may separate while removal from substrate. However, the film was easily picked by tweezers, handled in air and scooped in a petridish. This may probably due to the interaction between the layers which renders them inseparable. LATINA 2 and 3 were prepared in order to check the efficiency of single-drug loaded LATINA as compared to combinatorial drug loaded LATINA in IOP reduction.

The image of LATINA is shown in Fig. 1.

### 3.2. Scanning electron microscopy of LATINA

The SEM images of LATINA are depicted in Fig. 2. The images clearly show the multilayered structure of the nanosheet. Especially the control with alginate/chitosan shows an irregular and rough surface whereas the ones loaded with drug(s) are relatively smooth. LATINA 1 (10 layered) shows a thick layer of deposited drug whereas LATINA 1, 2 and 3 also show deposition of drug in the crevices of nanosheet. Although the thickness of the LATINA may improve its performance during IOP reduction, the extent of the adhesiveness remains to be seen in adhesive strength studies.

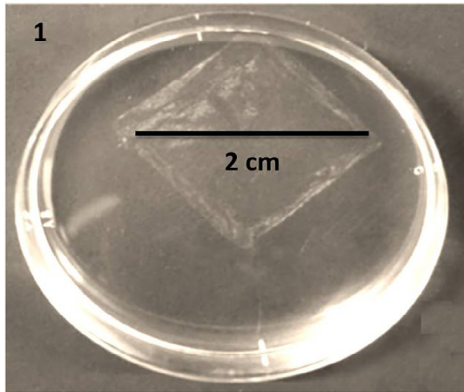


Fig. 1. LATINA nanosheet.

### 3.3. Atomic force microscopy of LATINA

The atomic force microscopy images are depicted in Fig. 3. The multilayered nanosheet is clearly visible. The thickness of the nanosheet is also determined; the values are given in table 1. The values are given as mean ± SD. The thickness is determined from the height profile of the image.

As seen and hypothesized, the control (figure a) had the least thickness (40 nm) and was seen to be filled with pits and crevices, although layered structure. The 10 layered LATINA 1 was the thickest as expected at 260 nm. These images also show that because of the drug coating, the pits and crevices seen in the control have become smooth (figures b, d, e). Figure c also show that the surface of 10 layered LATINA 1 was still rough despite drug coating because of probably being multilayered. The structure was seen to be amazingly flexible one while handling with tweezers. This would prove to be beneficial while transferring to cornea. Along with the flexibility, from the AFM images, a micropatterning of the nanosheets was visible too (figure a). The micropatterned nanosheet may facilitate efficient drug loading and delivery in the narrow ocular space. The pits and crevices seen in the surface of the control nanosheet may have significant potential to effectively encapsulate the drug(s).

### 3.4. Adhesive strength of LATINA

The adhesive strength studies are depicted in Fig. 4. This shows an interesting trend. The adhesive strength was determined using a micro-scratch tester and calculated depending upon the critical

Table 1  
Mean thickness of the nanosheets.

Sl. No.	Type of Nanosheet	Mean thickness in nm
1.	Control (chitosan/alginate)	50 ± 10
2.	LATINA 1 (3 layered)	70 ± 10
3.	LATINA 1 (10 layered)	260 ± 30
4.	LATINA 2	50 ± 20
5.	LATINA 3	55 ± 10

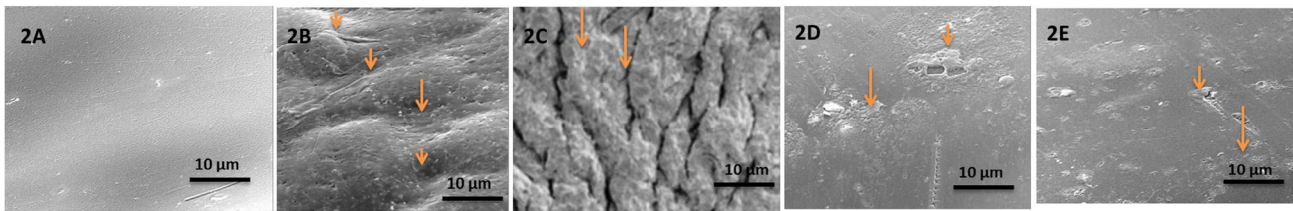


Fig. 2. Scanning electron Micrographs 2A. Alginate/Chitosan nanosheet (Control); 2B. LATINA 1 (3 Layered); 2C. LATINA 1(10 Layered); 2D. LATINA 2; 2E. LATINA 3. Note the multilayered coating of drugs in case of latanoprost/timolol.

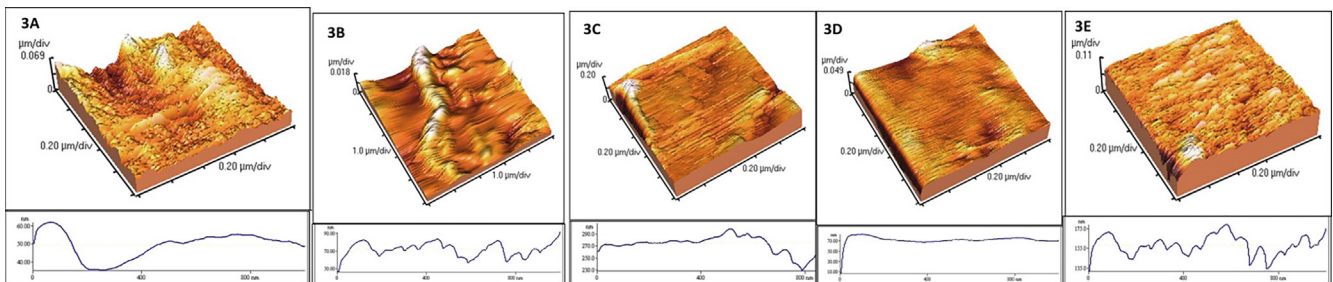


Fig. 3. Atomic Force microscopy Images 2A. Alginate/Chitosan nanosheet (Control); 2B. LATINA 1 (3 Layered); 2C. LATINA 1(10 Layered); 2D. LATINA 2; 2E. LATINA 3. The thickness was calculated from the height profile.

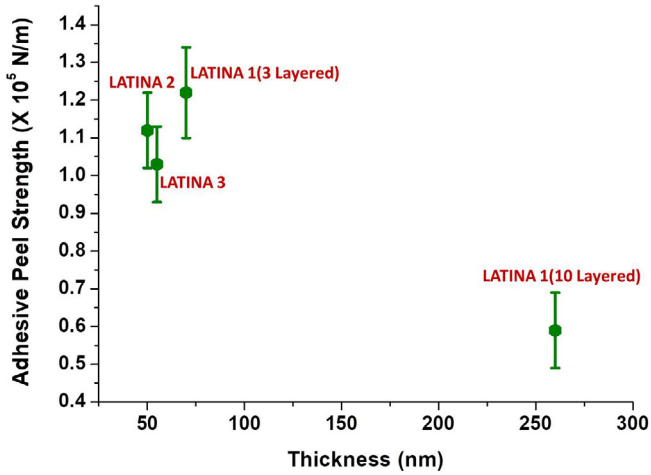


Fig. 4. Adhesive Peel strength of the LATINAs.

load just after detachment of the LATINAs from the SiO<sub>2</sub> substrate. It was seen that the adhesive strength was of 3 layered LATINA 1 (thickness 70 nm) was determined to be  $(1.22 \pm 0.12) \times 10^5$  N/m whereas that of LATINA 2 (thickness 50 nm) was determined to be  $(1.12 \pm 0.10) \times 10^5$  N/m. but surprisingly with increase in thickness of the LATINA, the adhesive strength decreased significantly. 10 layered LATINA 1 (thickness 260 nm) demonstrated adhesive strength of  $(0.59 \pm 0.10) \times 10^5$  N/m whereas LATINA 3 (thickness 55 nm) confirmed an adhesive strength of  $(1.03 \pm 0.10) \times 10^5$  N/m. This may be due to the fact that multilayers of the nanosheet were not detached in a step by step manner but removed together in one single step.

3.5. Burst studies of LATINA

The burst test studies results were shown in Fig. 5. As expected the burst pressure increased with increase in thickness of nanosheet. For bursting the control (thickness 40 nm), burst pressure required was  $1.8 \pm 0.10$  kPa. For 3 layered LATINA 1 (thickness 70 nm), burst pressure required was  $5.5 \pm 0.10$  kPa, 10 layered LATINA 1 (thickness 260 nm) required a burst pressure of  $19.1 \pm 5.10$  kPa, LATINA 2 (thickness 50 nm) required burst pressure of  $2.1 \pm 0.12$  kPa and LATINA 3 (thickness 55 nm) required a burst pressure of  $2.3 \pm 1.12$  kPa.

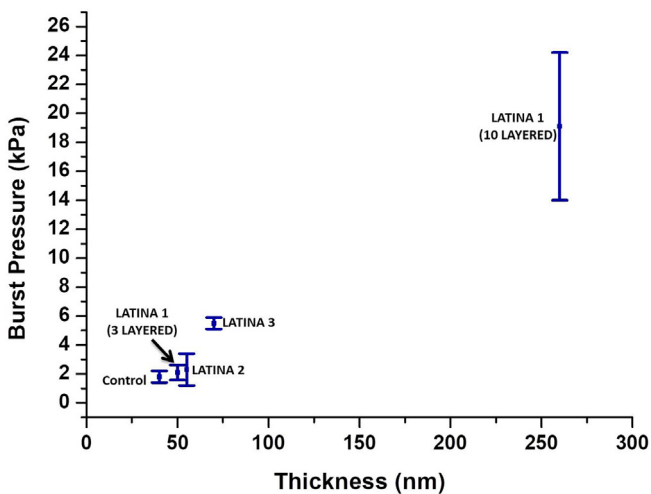


Fig. 5. Burst Pressure of the LATINAs and control.

3.6. In-vitro release studies

One  $2.5 \mu\text{g}/\text{cm}^2$  LATINA 1(3 layered) placed in saline released only 25% of latanoprost and timolol over a period of 48 hrs whereas from LATINA 2, 82% latanoprost was released in the first 48 h. Timolol showed an even greater release of 95% from LATINA 3 in first 48 h. This is depicted in Fig. 6. It was seen that the latanoprost/timolol released from the LATINA 1(10 layered) was even slower (12%). If the LATINA 1, 2 and 3 were faced upwards instead of towards the corneal surface, the release was slower but insignificant to be of any clinically relevant use.

3.7. Cytotoxicity studies of LATINA

The cytotoxicity results of LATINA are demonstrated in Fig. 7. Since chitosan and alginate are both biocompatible polysaccharides, the nanosheets were expected to be biocompatible. Moreover, latanoprost and timolol are being regularly used for ophthalmic formulations for treatment of glaucoma for quite a long time. It has been noted in quite a few studies that no serious ocular side effects have occurred in any patient after the application of latanoprost or timolol (Camras, 1996). Hence, there was almost no chances of LATINA being non-biocompatible/cytotoxic which was reinforced by the live/dead cell assay too (Fig. 8).

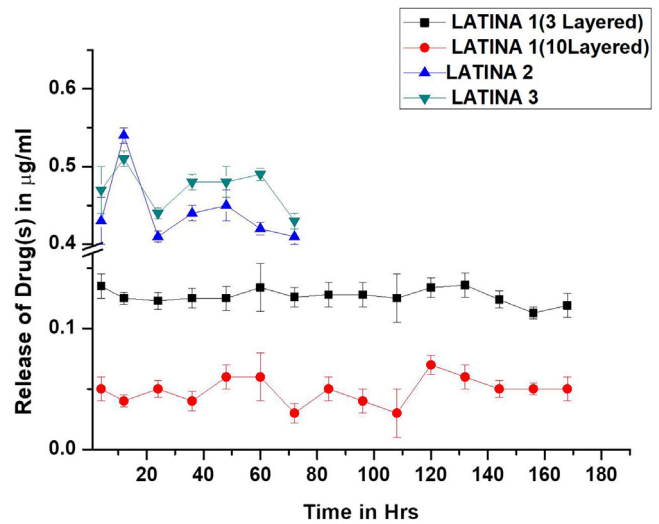


Fig. 6. In vitro releases of drug(s) from the LATINA.

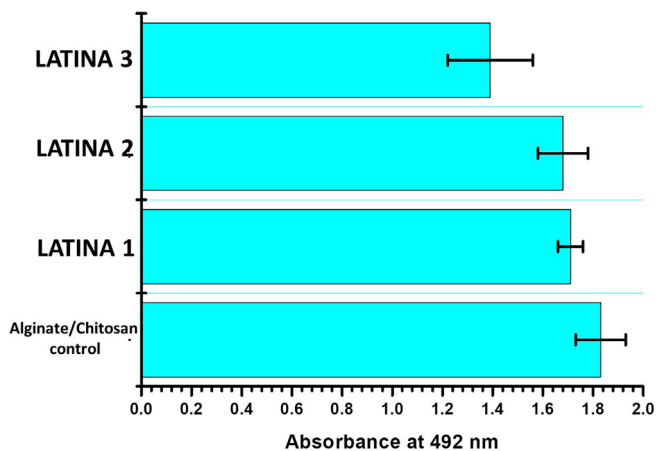


Fig. 7. Cytotoxicity of the control and LATINAs. Note the significance absence of difference in cell viability.



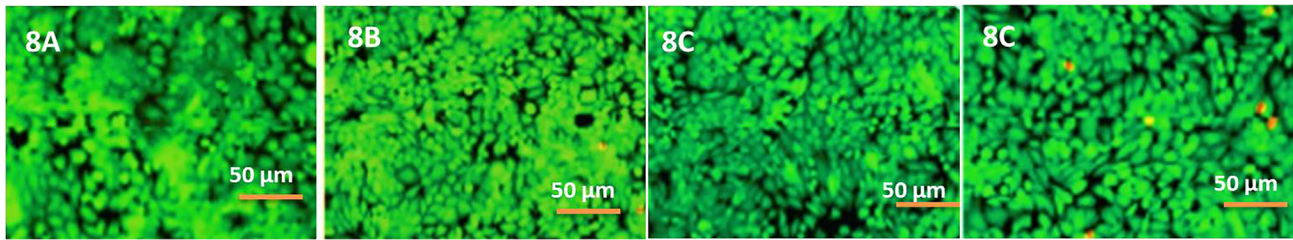


Fig. 8. Cytotoxicity of the LATINAs when treated with human ocular epithelial cell line. The green cells indicate live cells, whereas the red ones indicate dead cells.

### 3.8. In-vivo studies

Latanoprost (Perry et al., 2003) and timolol as individual ophthalmic formulations have to apply daily to reduce IOP during glaucoma. It was also seen that timolol may have to be applied twice daily sometimes in more acute conditions. Therefore the aim of our study was to create a potential drug delivery system which would deliver the drug(s) without having to be replaced or renewed daily. This should ideally be able to deliver a combination of drugs to the cornea without causing any adverse ocular events. Moreover the drug delivery system should be ideally biodegradable. Therefore we had prepared the LATINA which was proven to be biocompatible, strong and flexible with a sustained release of the combination of drugs.

The insertion of LATINA in the eye of the rats was seen in Fig. 9 through slit lamp microscopy images. The LATINA was smoothly inserted in the eye with blunt tweezers under a surgical microscope and afterwards with a few blinks, it was not even visible. The LATINA is a highly transparent structure and it spread smoothly and adhered tightly to the corneal surface. Initially after administration, few wrinkles were seen in the LATINA which immediately disappeared after rapid blinking.

#### 3.8.1. IOP reduction profile

The IOP reduction profile by the LATINA is tabulated in Table 2. IOP reduction by LATINA was seen after 1, 2, 4, 7 and 9 days after application and the initial reading 10 days before application was considered as the baseline.

#### 3.8.2. Estimation of eye scratching activity after application of LATINA

The scratching activities of eye of the Sprague-Dawley rats are depicted in Fig. 10. There were no noteworthy difference in scratching activity of eye with forelimbs and hind limbs between control eyes and eyes to which LATINA were applied. There was no significant difference between scratching movement of eye with forelimbs and hind limbs of control and sham-procedure. This may be due to the fact that LATINA are biocompatible and do not lead to any sort of irritation in the cornea. This particular study highlights the fact that biodegradable and biocompatible nanosheet may prove to be an ideal drug delivery system to most sensitive of organs too.

## 4. Conclusion

A devastating malady, glaucoma affects thousands of persons worldwide and severely hampers their lifestyle. As researchers uncover more information regarding glaucoma and its pathogenesis, more methods of novel treatments may be elucidated. In this study, a novel combinatorial drug loaded nanosheet therapy to reduce IOP during glaucoma has been detailed. This nanosheet is easy to prepare, does not cause any side-effects as other ophthalmic formulation and is biocompatible. This therapy may provide novel addition to the currently available IOP-lowering techniques. This novel LATINA may also relieve a subject from daily routine of administration of ophthalmic formulation. However, abundant caution must be exercised while moving away from the established treatments and undertaking novel ones. While it

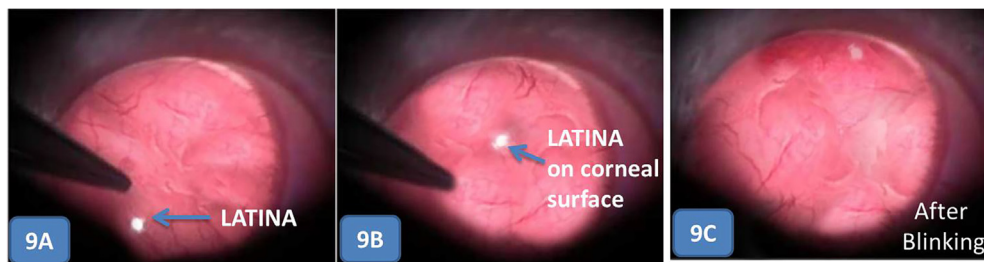


Fig. 9. Incorporation of LATINA on the corneal surface. Note that in 9C the LATINA is almost invisible after blinking.

Table 2  
Determination of IOP before and after application of LATINA;  $p > 0.01$ .

	Baseline IOP (mm of Hg)	Day 1 IOP (mm of Hg)	Day 2 IOP (mm of Hg)	Day 4 IOP (mm of Hg)	Day 7 IOP (mm of Hg)	Day 9 IOP (mm of Hg)
Control	23.3 ± 1.1	23.2 ± 1.1	23.0 ± 1.1	23.3 ± 1.0	23.1 ± 1.2	23.3 ± 1.0
LATINA 1 (3 layered)	23.1 ± 1.4	21.1 ± 2.1	19.4 ± 1.3	12.6 ± 1.2	12.3 ± 1.1	12.2 ± 1.2
LATINA 1 (10 layered)	23.2 ± 1.1	21.4 ± 1.1	21.5 ± 1.1	22.1 ± 1.1	22.4 ± 1.1	23.4 ± 1.1
LATINA 2	23.3 ± 1.4	17.8 ± 1.0	17.2 ± 1.0	17.8 ± 1.1	19.0 ± 1.8	19.1 ± 1.0
LATINA 3	23.3 ± 1.2	17.9 ± 2.1	17.8 ± 1.5	19.1 ± 1.3	20.0 ± 1.1	21.1 ± 1.6
Latanoprost	23.2 ± 1.1	16.8 ± 1.4	22.8 ± 1.5	23.1 ± 1.5	23.3 ± 1.1	23.1 ± 1.0
Timolol	23.1 ± 1.0	17.3 ± 1.4	21.9 ± 1.3	23.0 ± 1.1	23.2 ± 1.2	23.3 ± 1.4

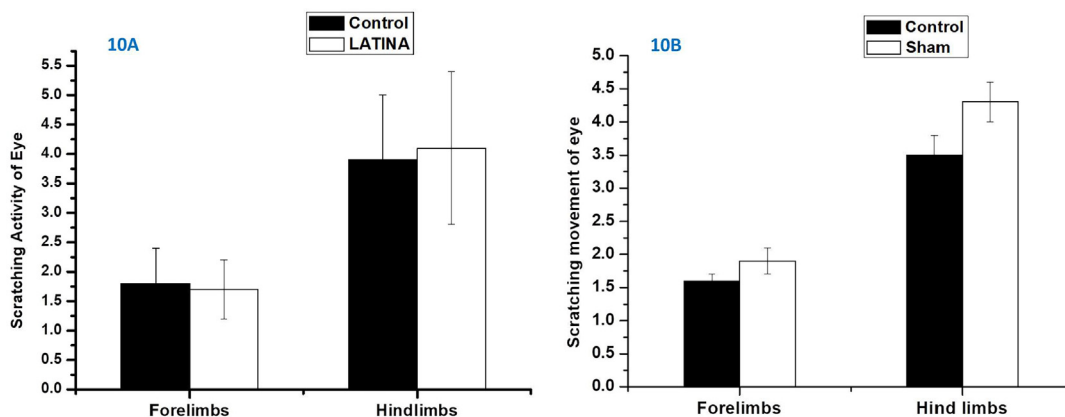


Fig. 10. Scratching movements of eye by fore and hind limbs after treatment with control/LATINA and control/sham. Students *t* test followed,  $P < 0.5$ .

may take some time to get approval for a new therapy to gain acceptance, more in-vivo research can definitely be taken up on nanosheet which acts a perfect drug delivery system which may benefit glaucoma-affected persons in future.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### References

- Alm, A., Villumsen, J., Törnquist, P., Mandahl, A., Airaksinen, J., Tuulonen, A., Marsk, A., Resul, B., Stjerschantz, J., 1993. Intraocular pressure-reducing effect of PhXA41 in patients with increased eye pressure: a one-month study. *Ophthalmology* 100, 1312–1317.
- Alm, A., Widengård, I., Kjellgren, D., Söderström, M., Friström, B., Heijl, A., Stjerschantz, J., 1995. Latanoprost administered once daily caused a maintained reduction of intraocular pressure in glaucoma patients treated concomitantly with timolol. *Br. J. Ophthalmol.* 79, 12–16.
- Ariga, K., Li, J., Fei, J., Ji, Q., Hill, J.P., 2016. Nanoarchitectonics for dynamic functional materials from atomic-/molecular-level manipulation to macroscopic action. *Adv. Mater.* 28, 1251–1286.
- Atabay, M., Sardroodi, J.J., Ebrahimzadeh, A.R., 2017. Adsorption and immobilisation of human insulin on graphene monoxide, silicon carbide and boron nitride nanosheets investigated by molecular dynamics simulation. *Mol. Simulat.* 43, 298–311.
- Bachu, R.D., Chowdhury, P., Al-Saedi, Z.H.F., Karla, P.K., Boddu, S.H.S., 2018. Ocular drug delivery barriers—role of nanocarriers in the treatment of anterior segment ocular diseases. *Pharmaceutics* 10, 28–59.
- Camras, C.B., 1996. Comparison of latanoprost and timolol in patients with ocular hypertension and glaucoma: a six-month, masked, multicenter trial in the United States. *Ophthalmology* 103, 138–147.
- Centinel, S., Montemagno, C., 2016. Nanotechnology and glaucoma. *Asia Pac. J. Ophthalmol.* 5, 70–78.
- Cho, J., Char, K., Hong, J.D., Lee, K.B., 2001. Fabrication of highly ordered multilayer films using a spin self-assembly method. *Adv. Mater.* 13, 1076–1078.
- Das, S., Neogi, S., Chainy, G.B.N., Guha, S.K., 2011. A Novel two-step procedure for plasma surface modification of low-density polyethylene for improved drug adhesion in intra uterine devices (IUDs). *J. Adhes. Sci. Technol.* 25, 151–167.
- Dou, Q.-L., Wei, Y.-Y., Gu, Y.-N., Zheng, H., 2017. Investigating the therapeutic effects of N-acetylcysteine decorated poly (L-lactic acid) nanoparticles on transfusion induced acute lung injury. *J. Biomater. Tissue Eng.* 7, 69–76.
- Dubey, A., Prabhu, P., Beladiya, K., Nair, N., Ghate, V., 2015. Development and investigation of timolol maleate and latanoprost combination liposomes for the treatment of glaucoma. *Int. Res. J. Pharm.* 6, 256–264.
- Forrest, J.A., Dalnoki-Veress, K., 2001. The glass transition in thin polymer films. *Adv. Colloid Interface Sci.* 94, 167–195.
- Fujie, T., Matsutani, N., Kinoshita, M., Okamura, Y., Saito, A., Takeoka, S., 2009. Adhesive, flexible, and robust polysaccharide nanosheets integrated for tissue-defect repair. *Adv. Funct. Mater.* 19, 2560–2568.
- Fujie, T., Okamura, Y., Takeoka, S., 2007. Ubiquitous transference of a free-standing polysaccharide nanosheet with the development of a nano-adhesive plaster. *Adv. Mater.* 19, 3549–3553.
- Gong, T., Su, X.-T., Xia, Q., Wang, J.-G., 2017. Biodegradable combinatorial drug loaded pH-sensitive liposomes for enhanced osteosarcoma therapeutics. *J. Biomater. Tissue Eng.* 7, 952–961.
- Hotehama, Y., Mishima, H., Kitazawa, Y., Masuda, K., 1993. Ocular hypotensive effect of PhXA41 in patients with ocular hypertension or primary open-angle glaucoma. *Jpn. J. Ophthalmol.* 37, 270–274.
- Jiang, C., Markutsya, S., Tsukruk, V.V., 2004. Compliant, robust, and truly nanoscale free-standing multilayer films fabricated using spin-assisted layer-by-layer assembly. *Adv. Mater.* 16, 157–161.
- Kashiwagi, K., Ito, K., Haniuda, H., Ohtsubo, S., Takeoka, S., 2013. Development of latanoprost-loaded biodegradable nanosheet as a new drug delivery system for glaucoma. *Invest. Ophthalmol. Vis. Sci.* 54 (8), 5629–5637.
- Kim, N.J., Harris, A., Gerber, A., Tobe, L.A., Amireskandari, A., Huck, A., Seisky, B., 2014. Nanotechnology and glaucoma: a review of the potential implications of glaucoma nanomedicine. *Br. J. Ophthalmol.* 98, 427–431.
- Komachi, T., Sumiyoshi, H., Inagaki, Y., Takeoka, S., Nagase, Y., Okamura, Y., 2017. Adhesive and robust multilayered poly (lactic acid) nanosheets for hemostatic dressing in liver injury model. *J. Biomed. Mater. Res. B. Appl. Biomater.* 105, 1747–1757.
- Nair, K.S., Cosma, M., Raghupathy, N., Sellarole, M.A., Tolman, N.G., de Vries, W., Smith, R.S., John, S.W., 2016. YBR/Eij mice: a new model of glaucoma caused by genes on chromosomes 4 and 17. *Dis. Model. Mech.* 9, 863–871.
- Natarajan, J.V., Ang, M., Darwitan, A., Chattopadhyay, S., Wong, T.T., Venkatraman, S. S., 2012. Nanomedicine for glaucoma: liposomes provide sustained release of latanoprost in the eye. *Int. J. Nanomed.* 7, 123.
- Natarajan, J.V., Darwitan, A., Barathi, V.A., Ang, M., Htoon, H.M., Boey, F., Tam, K.C., Wong, T.T., Venkatraman, S.S., 2014. Sustained drug release in nanomedicine: a long-acting nanocarrier-based formulation for glaucoma. *ACS Nano* 8, 419–429.
- Okamura, Y., Kabata, K., Kinoshita, M., Saitoh, D., Takeoka, S., 2009. Free-standing biodegradable poly (lactic acid) nanosheet for sealing operations in surgery. *Adv. Mater.* 21, 4388–4392.
- Okamura, Y., Nagase, Y., Takeoka, S., 2015. Patchwork coating of fragmented ultrathin films and their biomedical applications in burn therapy and antithrombotic coating. *Materials* 8, 7604–7614.
- Perry, C.M., McGavin, J.K., Culy, C.R., Ibbotson, T., 2003. Latanoprost: an update of its use in glaucoma and ocular hypertension. *Drugs Aging* 20, 597–630.
- Ricotti, L., Taccola, S., Pensabene, V., Mattoli, V., Fujie, T., Takeoka, S., Menciaci, A., Dario, P., 2010. Adhesion and proliferation of skeletal muscle cells on single layer poly (lactic acid) ultra-thin films. *Biomed. Microdevices* 12, 809–819.
- Sleath, B., Blalock, S.J., Carpenter, D.M., Sayner, R., Muir, K.W., Slota, C., Lawrence, S. D., Giangiacomo, A.L., Hartnett, M.E., Tudor, G., Goldsmith, J.A., Robin, A.L., 2015. Ophthalmologist-patient communication, self-efficacy, and glaucoma medication adherence. *Ophthalmology* 122, 748–754.
- Stjerschantz, J., 1992. Phenyl-substituted prostaglandin analogs for glaucoma treatment. *Drugs Future* 17, 691–704.
- Stone, J.L., Robin, A.L., Novack, G.D., Covert, D.W., Cagle, G.D., 2009. An objective evaluation of eyedrop instillation in patients with glaucoma. *Arch. Ophthalmol.* 127, 732–736.
- Vanelli, M., Pedan, A., Liu, N., Hoar, J., Messier, D., Kiarsis, K., 2009. The role of patient inexperience in medication discontinuation: a retrospective analysis of medication nonpersistence in seven chronic illnesses. *Clin. Ther.* 31, 2628–2652.
- Weng, Y., Liu, J., Jin, S., Guo, W., Liang, X., Hu, Z., 2017. Nanotechnology-based strategies for treatment of ocular diseases. *Acta. Pharm. Sin.* B 7, 281–291.
- Wentz, S.M., Kim, N.J., Wang, J., Amireskandari, A., Siesky, B., Harris, A., 2014. Novel therapies for open-angle glaucoma. *F1000Prime Rep.* 6, 102. <https://doi.org/10.12703/P6-102>.
- Wong, T.T., Novack, G.D., Natarajan, J.V., Ho, C.L., Htoon, H.M., Venkatraman, S.S., 2014. Nanomedicine for glaucoma: sustained release latanoprost offers a new therapeutic option with substantial benefits over eyedrops. *Drug. Deliv. Transl. Res.* 4, 303–309.