TOURO COLLEGE & UNIVERSITY SYSTEM

The Science Journal of the Lander College of Arts and Sciences

Volume 10
Number 2 *Spring 2017*

2017

Are Contact Lenses an Effective Vehicle for Ocular-Disease Drug Delivery?

Chana Haimowitz
Touro College

Are Contact Lenses an Effective Vehicle for Ocular-Disease Drug Delivery?

Chana Haimowitz

Chana Haimowitz graduated with a BS in Biology in January 2017 and will attend SUNY College of Optometry in August 2017.

Abstract

Due to numerous drawbacks with current modes of treatment for various ocular diseases, researchers are synthesizing drug dispensing contact lenses. The lenses will contribute to greater bioavailability of the drug, the minimization of negative side effects, and increased patient compliance. As treatment for glaucoma, in vivo studies have been conducted with latanoprost, timolol maleate, and brimonidine tartrate-eluting lenses, and have succeeded in reducing intraocular pressure to desired values (Ciolino et al., 2016), (Schultz and Mint, 2002). As treatment for fungal keratitis, in vitro studies prove that econazole and natamycin-eluting contact lenses have been successful in killing 100% of fungi for sustained periods of time (Ciolino et al., 2011), (Phan et al., 2013). Finally, for allergic conjunctivitis, contact lenses containing nanoparticles of prednisolone have been synthesized and demonstrate effective drug-releasing capabilities (ElShaer et al., 2016).

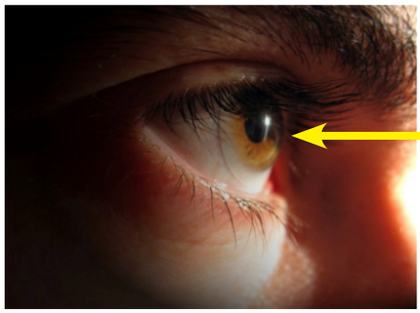
Introduction

Current methods of treatment for various ocular ailments include both oral medications and topical eye drops. There are significant downsides to both. Orals are often not the first line of treatment both because, they take a circuitous route to the eye and cause many more negative systemic side effects (Kim, et al., 2014). Next, in the case of eye drops, there are multiple barriers to overcome. First, much of the dispensed eye drop is inhibited by pre-corneal factors which include nasolacrimal drainage, tearing, and blinking. These factors significantly lower the bioavailability of the medication. Research indicates that only a fraction of the precious medication, a mere one percent to seven percent, reaches its required destination, thereby reducing the drug's effectiveness (Schultz and Mint, 2002). Furthermore, the drops are often administered by the patient, and sometimes are required multiple times a day. This commonly leasds to low patient compliance, and doses are frequently forgotten or skipped purposely (Ciolino et al., 2011). Given the above, there exists an impetus to develop alternate methods of delivering ocular medications, thus enabling effective treatment. Researchers are currently working on developing a contact lens that will also dispense nanoparticles of medication directly into the eye while correcting refractive error. In patients who don't have refractive error, the contact lenses can simply be worn for the purpose of delivering the needed medication into their eyes. The use of contact lenses for ocular drug delivery can solve many of the issues associated with eye drops. First, the space created by the lens with the cornea has limited tear mixing, and potentially a greater amount of contact time between the drug and the cornea.

This causes greater bioavailability. Additionally, there is an added benefit of eliminating the need for multiple doses a day, which will increase the amount of patient compliance. Under ideal kinetics, the drug will release in a time dependent manner, extending the therapeutic effects of one dose (Phan et al., 2014). Research with a drug dispensing contact lens (DDCL), is currently underway for a number of ocular conditions. In this work specifically, a DDCL for the diseases of glaucoma, fungal keratitis, and hay fever are discussed.

Glaucoma

Glaucoma, a group of conditions that damages the eye's optic nerve, usually results from increased intraocular pressure (IOP) which can result in vision loss and blindness. The two main forms are open-angle glaucoma and angle-closure glaucoma. Both forms, involve clogging of the eye's drainage canals, leading to elevated ocular pressures and subsequent nerve damage. In open-angle glaucoma this leads to a gradual increase in IOP because, the angle between the iris and cornea is wide and open. In angle-closure glaucoma there is a sudden



increase in IOP because, the angle between the iris and cornea is either very narrow or closed. Current methods of treatment include surgery, oral medications, and eye drops, depending on the classification and extent of the disease. When surgery is warranted however, it usually does not resolve the increased IOP completely and generally a regimen of eye drops are prescribed as well post operatively. Additionally, surgery can cause negative side effects including, cataract formation, inflammation, ocular infections, corneal issues, and low IOP. Negative side effects caused by oral medications can include irritation, stinging, redness, blurred vision, itchiness, low blood pressure, fatigue, shortness of breath, headaches, dry mouth, frequent urination, upset stomach, and memory problems, depending on the class of drug prescribed. In general, topical eye drops are the first line of treatment. However, due to the issues posited above, a contact lens that can dispense anti-glaucoma drugs is currently being developed.

Research for Anti-Glaucoma Drug Dispensing Contact Lens

Joseph B. Ciolino, MD, at the Massachusetts Eye and Ear, conducted an in vivo study on the effectiveness of a Latanoprost-dispensing contact lens for female monkeys with induced glaucoma. Latanoprost is currently on the market as a topical anti-glaucoma eye drop. It belongs to a class of anti-glaucoma medications knows as prostaglandin analogues, which work to lower the IOP by increasing uveoscleral flow (although more recently research suggests that it may occur through a trabecular pathway) and it is prescribed for cases of open-angle glaucoma (Lindén and Alm, 1999, Winkler and Fautsch, 2013). In Ciolino's research, a thin latanoprost-polymer film was introduced into a methafilcon hydrogel contact lens. Both a low-dose contact lens (CL), and a high-dose CL were synthesized. The intraocular pressure of the glaucomatous monkeys was monitored after a period of the following cases:

1. Treatment with Latanoprost eye drops
2. Treatment with CL-low
3. Treatment with CL-high
4. No treatment

The results demonstrated that the latanoprost eye drops succeeded in reducing IOP approximately 5 mmHg, the CL-lo by about 6.5 mmHg, and CL-hi by about 11 mmHg.

Evidently, "Sustained delivery of latanoprost by contact lenses is at least as effective as delivery with daily latanoprost ophthalmic solution. More research is needed to determine the optimal continuous-release dose that would be well tolerated and maximally effective. Contact lens drug delivery may become an option for the treatment of glaucoma and a platform for ocular drug delivery (Ciolino et al., 2016)"

Additional anti-glaucoma contact lenses that are currently being researched, synthesized, and patented are those that contain timolol maleate or brimonidine tartrate within a polymeric hydrogel. Timolol maleate belongs to a class of anti-glaucoma drugs known as beta-adrenergic blockers and brimonidine tartrate is an alpha agonist. Both are prescribed for cases of open-angle glaucoma. Specifically, in the case of beta blockers, systemic side effects can be pretty severe such as, cardiac arrhythmias, bronchospasm, and stroke and is therefore prescribed based on a patient's complete medical history. The aim of the study was to develop contact lenses that maintain normal hydration and comfort, and will dispense lower doses of drug for extended periods of time. This will lead to increased patient compliance, decreased negative side effects, and efficacious treatment (Schultz and Mint, 2002).

Etafilcon contact lenses (hydrogels) were washed in a saline solution and briefly dried. Then they were immersed in either a dilute solution of brimonidine tartrate (0.02%), or a dilute solution of timolol maleate (0.05%). (Topical ophthalmic solutions of the above drugs are commercially available as 0.2% solutions for brimonidine, 0.25% for timolol, and 0.5% for timolol ophthalmic gel forming solution). The lenses were subsequently tested on multiple patients as a replacement for their current regimens of eye drops. Instead of the patient administering their daily eye drop, he wore the contact lens for 30 minutes each day. In all cases, this method allowed for IOP to remain below the necessary value of 20 mmHg, with no evidence of ocular toxicity.

An additional study was conducted on glaucomatous beagle dogs. NIGHT & DAY™ silicone hydrogel contact lenses were immersed in timolol and phosphate buffered saline solution. Then, one lens was inserted into one of the dog's eyes, while the other eye served as the control, and no lens was inserted. The lenses with similar dosing to timolol eye drops led to an IOP reduction of about 5 mmHg (which is slightly greater than the IOP reduction resulting from timolol eye drops). However, lenses with a third drug loading as the eye drops led to a similar reduction in intraocular pressure, suggesting increased bioavailability. Finally, the eye without the contact lens remained unaffected by its proximal lens, which suggests reduction in systemic absorption of the drug released by the lens (Peng et al., 2012).

Fungal Keratitis

Fungal keratitis is an infection of the cornea (the clear, round dome covering the eye's iris and pupil) which causes pain, reduced vision, light sensitivity, and tearing or discharge from the eye. Resulting from infection from contact lens use, or from injury to the eye, fungal keratitis usually develops very quickly, and if left untreated, can cause blindness (Boyd, 2015). Fungal keratitis is also prevalent in tropical and subtropical climates (Ciolino et al., 2011).

Current treatment options for fungal keratitis vary depending on the severity of the condition. Topical eye drops are often the first line of treatment (Ciolino et al., 2011). Once again the above drawbacks to eye drops are present:

> "The failures of topical antimycotic treatments may be related to the limitations of eye drops as a form of drug delivery. Eye drops generate a transiently high concentration on application followed by a short period of effective therapeutic concentration and then a prolonged period of underdose. Furthermore, each drop is diluted

> and washed away by reflex tearing and dispersed by blinking. As a consequence, only 1% to 7% of drug in a drop is absorbed in the eye. The cornea absorbs only a fraction of this dose, in part due to the tissue's short contact time with the topical drops (Ciolino et al., 2011)."

Currently there is only one drug available on the market, natamycin, as a topical ophthalmic antifungal. However, this drug specifically is shown to have poor corneal penetration and is mainly effective with superficial corneal infections caused by Fusarium species (Singh, 2015). Depending on the severity and identity of the disease, often subconjunctival injections of an antifungal agent are prescribed and the dosage times are not infrequent, (twice every hour for the first 24 hours, then once every hour for the next 24 hours etc). "Successful antifungal therapy for fungal keratitis requires frequent drug administration for prolonged periods (ie, at least 12 weeks) (Singh, 2015)." Sometimes antifungals in an oral form are prescribed. However, 15 to 27 percent of patients with fungal keratitis require surgical intervention (Boyd, 2015). Even after surgery, a course of topical drops is often prescribed as well. Finally, surgery is not effective in all cases, and a patient may be rendered significantly visually impaired (Singh, 2015).

Research for an Anti-Fungal Contact Lens

The ineffectiveness of the topical regiment arises from low penetrance of the drug to the corneal epithelium as well as inadequate contact time between drug and tissue. Additionally, low patient compliance is common due to the frequency with which the drug needs to be administered. A contact lens that dispenses antifungal particles could resolve all these issues. A prototype antifungal contact lens (Ciolino et al., 2011) was synthesized using the following method:

Econazole, an antifungal drug, was added to a film of poly (lactic-co-glycolic) acid (PLGA). PLGA is desirable because of its biocompatibility and biodegradability, and its effectiveness at controlling drug release kinetics. Various film sizes were synthesized and all were encapsulated into polyhydroxymethacrylate (pHEMA), a common contact lens material. Contact lenses were synthesized with different concentrations of econazole. A control lens was created as well which contained the PLGA film inside the pHEMA hydrogel without the econazole. The contact lenses were tested against the fungus C. albicans, a common agent of fungal keratitis. First the lenses were placed directly onto a rich medium, a culture plate containing 1 mL of the Candida suspension. After a number of cycles of incubation and refreshing the medium, the culture was diluted, incubated, and counted for viable colonies. This was done to determine the effect of the contact lenses in direct contact with the fungi. The lenses were also tested for their drug-releasing capabilities. The testing was conducted by immersing the lenses in a yeast nitrogen base medium and incubated. Then they were immersed in fresh medium every 24 hours. The yeast nitrogen base drug release medium was collected at different intervals, and diluted with new medium containing C. albicans. Once again after a period of dilution and incubation, the suspension was plated and counted for viable colonies.

The results showed that both methods were capable of killing 100% of fungi for extended intervals. The release medium which contained contact lenses with 16 mg of econazole (PLGA-16) killed 100% of fungi for 21 days! The mediums from contact lenses, containing lower concentrations of econazole, killed fungus for shorter amounts of time. In the cases where the contact lenses came directly in contact with the fungal suspension, 100% of fungi were killed for 8 to 10 days (with PLGA-16). Studies show that C. albicans is more difficult to kill than Fusarium species. Therefore if Candida was killed by econazole, Fusarium should be as well (Ciolino et al., 2011). (Currently econazole is not FDA approved for ophthalmic use, although many ophthalmologists would prefer to treat fungal infections with something other than Natamycin, currently the only available drug).

Contact lenses that could elute the drug natamycin have also been synthesized (Phan et al., 2013). The study focused specifically on manipulating various contact lens materials. Hydrogels were composed of:

1. 100 % pHEMA,

2. 85% pHEMA and 15% [Tris(trimethylsiloxy)silyl]-propyl methacrylate (TRIS)

3. 75% pHEMA and 25% TRIS

4. N,N-dimethylacrylamide (DMAA),

5. 85% DMAA and 15% TRIS

6. 75% DMAA and 25% TRIS

The lenses were monitored by their uptake and release of two forms of natamycin. The first form was Natamycin dissolved in deionized water, and the second form was Natamycin encapsulated within poly(D,L-lactide)-dextran nanoparticles. Results indicated that the optimal materials to use were those containing DMAA. Furthermore, all gels had a greater uptake with the nanoparticles of natamycin versus natamycin alone. Finally, the release of natamycin within nanoparticles was greater than the natamycin alone. Also, the first hour of release was noteworthy.

The lenses with regular natamycin released 28-82% within the first hour. In the nanoparticle lenses this was reduced to 21-54% (Phan et al., 2013).

Hay Fever and Allergic Conjunctivitis

"Spring allergies are triggered as trees start blooming and billions of pollen grains are released into the air, causing susceptible individuals to develop allergic rhinitis… In these patients, the pollen causes degranulation of mast cells, which contain inflammatory mediators, ie, histamine and other allergy-causing chemicals. This process is clinically represented by sneezing; red, tearing eyes; postnasal drip; sinus headaches; feelings of sinus fullness; and itchy, scratchy throat (Medscape, 2016)."

As the conjunctiva of the eye is a mucosal membrane, it too is subjected to the inflammatory responses of allergic rhinitis. Per the CDC (Center for Disease Control and Prevention), approximately 19 million adults and another 6 million children suffer from hay fever in the U.S. alone. For the ocular symptoms of rhinitis, also known as allergic conjunctivitis, eye drops containing anti-histamines and/or mast cell stabilizers can be prescribed or recommended. Another class of treatments are corticosteroids and glucocorticoids, which also work to reduce the allergic reactions which affect the eye and relieve the negative symptoms. These can be prescribed as an ophthalmic suspension for topical use. Once again due to inhibitive pre-corneal factors, an alternate drug delivery mechanism is currently being researched in the form of a drug dispensing contact lens.

Research for DDCLs for Allergic Conjunctivitis

The Kingston University London conducted an in vitro experiment to synthesize a contact lens that could dispense nanoparticles of a synthetic glucocorticoid, prednisolone, into the eyes of patients with allergic conjunctivitis. (Currently prednisolone is on the market as prednisolone acetate ophthalmic suspension for topical use.) The main purpose of the experiment was to consider the effects of the encapsulated drug on the contact lenses' functionality and safety as well as the drug's bioavailability (ElShaer et al., 2016).

Prednisolone nanoparticles (PNP) were synthesized using an emulsion-solvent evaporation method. The experiment was designed to maximize three key nanoparticle features: small particle size (increased surface area/bioavailability), highest encapsulation efficiency, and maximum surface charge (no coagulation of particles; increases stability). To obtain the smallest particle size, four variables were manipulated: PLGA (poly-lactic-co-glycolic acid), PVA (polyvinyl alcohol), API (amount of prednisolone used), and homogenization time. Through optimization of these components a particle size of about 295 nm was obtained. To form the contact lens molds, HEMA (2-hydroxymethacrylate), MAA (methacrylic acid) and a small amount of EGDMA (ethylene glycol dimethacrylate) were mixed together along with the PNPs. These four hydrogel materials were allowed to polymerize thermally for 4 hours at 80°C in molds of polypropylene.

The in vitro drug release pattern of the contact lens with 0.4 grams of PNP was observed to be a two-phase process: an initial burst, followed by a period of slower release. The lens was placed in a release medium of phosphate buffered saline for 24 hours. 10.8% of drug was released in that time. The slow release of the drug can be due to the need for the drug to get past its nanoparticle barrier and through the contact lens as well. One of the issues with eye drops is that all of the drugs are released within a few hours. Nanoparticles of medication embedded in contact lenses can provide a longer lasting therapeutic regimen (ElShaer et al., 2016).

Method of Drug Release from Contact Lens

Although some studies for drug-eluting contact lenses pre-soak the contact lenses in drug, to allow for eventual diffusion into the eye, to achieve a more controlled method of release, other methods are being researched. The human tear film contains an enzyme called lysozyme. In a study on anti-glaucoma contact lenses, timolol maleate was encapsulated in nanodiamond (ND) particles. The NDs were coated in both polyethyleneimine (PEI) and chitosan. Chitosan is an enzyme-cleavable polysaccharide and PEI enables a more effective cleavage. The drug release of these impregnated lenses was monitored in vitro. In the absence of lysozyme, no release of timolol maleate was detected. In the presence of lysozyme the lens released 9.41 micrograms in 24 hours (Kim et al., 2014).

Addition of Drug to Contact Lens Material and Subsequent Hydration and Oxygen Permeability

This feature was monitored in the prednisolone study cited above. Contact lenses lacking the PNPs had an average hydration of about 36%. Lenses containing a smaller volume of drug nanoparticles (0.2 g) had a decreased hydration by about 31%, whereas the lenses with a higher volume of PNPs (0.4 g) had a further reduction in hydration to about 30.5%.

Surface wettability determines comfort of the lens, and was measured as well. A good surface wettability is identified by a contact angle less than 90 °C. Unmodified lenses have a contact angle of 85 °C. The prednisolone encapsulated lenses had further reduced angles which should increase ocular comfort (ElShaer et al., 2016). Similarly, contact lenses containing nanodiamond particles of timolol maleate demonstrated acceptable hydration values (Kim et al., 2014).

Transparency/light Transmission Capability of Drug-Impregnated Lens

Ideally a contact lens should have a light transmittance of above 90%, so vision is unobstructed. The control contact lenses (lacking PNP) in the prednisolone study had a high transparency of 94.5%. The lenses containing 0.2 grams and 0.4 grams of PNP had a reduced transparency of 86.23% and 83.1% respectively. However, this amount contributes to low or no opacity, and as long as the correct amount of nanoparticle is added to the lens, vision should not be compromised (ElShaer et al., 2016). Similarly, addition of nanodiamond particles of timolol maleate to a pHEMA lens did not cause any discernable changes to the lens' optical clarity. The lens with a higher concentration of NDs maintained a transmittance of 84.5% (Kim et al., 2014).

Dimensions/measurements of Drug-Eluting Contact Lenses Compared to Commercially Available Lenses

In the study done on antifungal contact lenses, when synthesizing the econazole-laden lenses, parameters of an 8.05 base curve and a 15.5 mm diameter were measured, which are consistent with commercially available lenses (Ciolino et al., 2011).

Preservation of Contact Lens through Lyophilization (to prevent drug elution/ degradation) Effect on Lens Capability

Depending on the method used to impregnate the lenses with drug, there exists a risk of the drug eluting out of the lens during storage. In order to combat this, anti-fungal contact lenses were lyophilized, a preservation process involving the freeze drying of a substance and subsequent removal of water by a vacuum causing the water to go from an ice state directly to a gaseous one. The fungicidal activity of the lyophilized lens was then assessed and found to be intact, although the duration of its effectiveness was reduced by 1 to 2 days (Ciolino et al., 2011).

Risk Factors and Drawbacks Associated with a DDCL

Although there is a lot of potential is this innovative drug delivery system, several potential downsides should be noted. There are many consumers who do not wear contact lenses because they find them uncomfortable or haven't found the proper fit. Others do not wear contact lenses because they have no refractive error and would thus need a special fitting session just to wear a short-term lens. Additionally, glaucoma often affects the geriatric population. Individuals of this population could also have difficulty inserting and removing the lenses, however this issue could be aided by an eye-care professional. These factors could potentially minimize the market for such lenses. Another problematic feature involves the drug-eluting property of the lenses. Once removed by the patient any remaining drug may continue to diffuse out. In the case of anti-fungal drugs, this could have an effect on the development of resistant strains while in the case of other drugs this may simply pose as a hazard for children. (It should be noted though that with lenses controlled by lysozyme presence this undesired drug-elution may be minimized).

Conclusion and Further Applications

Contact lenses for the treatment of glaucoma, fungal keratitis, and allergic conjunctivitis have been synthesized and demonstrate much potential in effective treatment. However, the lenses are far from having a clinical relevance. Much more animal and human testing is required prior to the necessary FDA-type approvals. Although in this paper glaucoma, fungal keratitis, and allergic conjunctivitis were discussed, research is also underway for additional ocular conditions such as, chronic dry eye and bacterial infections (Legett, 2009), (ElShaer et al., 2016). Additionally, the studies are working on embedding various drug nanoparticles into lenses without obstructing optical transparency. Potentially, instead of drugs, various ocular-necessary vitamins and supplements can serve as the embedded nanoparticle. As salt is iodized to promote proper thyroid function, perhaps macular degeneration could be prevented by infusing contact lenses with nanoparticles of lutein and zeaxanthin, two nutrients vital to a healthy macula.

References

Boyd, Kierstan, American Academy of Ophthalmology, March 1, 2015. What is Fungal Keratitis. Available at: https://www.aao.org/eye-health/diseases/what-is-fungal-keratitis. Accessed December 2016.

Centers for Disease Control and Prevention. Allergies and Hay Fever, July 6, 2016. Available at: https://www.cdc.gov/nchs/fastats/allergies.htm. Accessed January, 2017.

Ciolino Joseph B, Ross Amy E, Tulsan Rehka, Watts Amy C, Wang Rong-Fang, Zurakowski David, Serle Janet B, Kohane Daniel S. Latanoprost-Eluting Contact Lenses in Glaucomatous Monkeys. Ophthalmology, Volume 123, Issue 10, October 2016, Pages 2085-2092. [PubMed]

Ciolino Joseph B, Hudson Sarah P, Mobbs Ashley N, Hoare Todd R, Iwata Naomi G, Fink Gerald R, Kohane Daniel S. A Prototype Antifungal Contact Lens. Invest Ophthalmol Vis Sci. 2011 Aug; 52(9): 6286-6291. [PubMed]

ElShaer Amr, Mustafa Shelan, Kasar Mohamad, Thapa Sapana, Ghatora Baljit, Alany Raid G, Nanoparticle-Laden Contact Lens for Controlled Ocular Delivery of Prednisolone: Formulation Optimization Using Statistical Experimental Design, Pharmaceutics. 2016 Jun; 8(2): 14. [PubMed]

Glaucoma Research Foundation. Glaucoma Medications and their Side Effects, September 2016. Available at: http://www.

glaucoma.org/gleams/glaucoma-medications-and-their-side-effects.php. Accessed December, 2016.

Glaucoma Research Foundation, 2016. Types of Glaucoma. Available at: http://www.glaucoma.org/glaucoma/types-of-glaucoma.php. Accessed December 2016.

Kim HJ1, Zhang K, Moore L, Ho D. Diamond nanogel-embedded contact lenses mediate lysozyme-dependent therapeutic release. ACS Nano. 2014 Mar 25;8(3):2998-3005. doi: 10.1021/nn5002968. Epub 2014 Feb 12. [PubMed]

Legett, Hadley, Contact High: Lenses that Deliver Drugs, July 21, 2009. Available at: https://www.wired.com/2009/07/druglens/. Accessed January, 2017.

Lindén C1, Alm A., Prostaglandin analogues in the treatment of glaucoma. 1999

May;14(5):387-98. [PubMed]

Medscape, Aug 10, 2016. Allergic Conjunctivitis. Available at: http://reference.medscape.com/features/slideshow/allergic-reactions. Accessed December ,2016.

National Institute of Health: National Eye Institute. Facts About Glaucoma. Available at: https://nei.nih.gov/health/glaucoma/glaucoma_facts. Accessed November, 2016.

Phan CM, Hui , Subbaraman L, Jones L (2014) Insights to Using Contact Lenses for Drug Delivery. January 2, 2014. Journal of Clinical and Experimental Pharmacology 4:145. doi: 10.4172/2161-1459.1000145

Phan CM1, Subbaraman L, Liu S, Gu F, Jones L. In vitro uptake and release of natamycin Dex-b-PLA nanoparticles from model contact lens materials. J Biomater Sci Polym Ed. 2014;25(1):18-31. doi: 10.1080/09205063.2013.830914. Epub 2013 Sep 6. [PubMed]

Peng CC1, Ben-Shlomo A, Mackay EO, Plummer CE, Chauhan A. Drug delivery by contact lens in spontaneously glaucomatous dogs. Curr Eye Res. 2012 Mar;37(3):204-11. doi: 10.3109/02713683.2011.630154. [PubMed]

Schultz, Clyde Lewis and Mint, Janet M. 2002. Patents: Drug Delivery System for Antiglaucomatous Medication, US 6410045 B1. Available at: http://www.google.com/patents/US6410045. Accessed November, 2016.

Singh, Daljit, Fungal Keratitis Treatment and Management. May 20, 2015. Medscape. Available at: http://emedicine.medscape.com/article/1194167-treatment. Accessed November, 2016.

Winkler NS1, Fautsch MP. Effects of prostaglandin analogues on aqueous humor outflow pathways. J Ocul Pharmacol Ther. 2014 Mar-Apr;30(2-3):102-9. doi: 10.1089/jop.2013.0179. Epub 2013 Dec 20. [PubMed]