ABSTRACT

Modern biological research has produced increasing number of promising therapeutic possibilities for medical treatment. These include for example growth factors, monoclonal antibodies, gene knockdown methods, gene therapy, surgical transplantations and tissue engineering. Ocular application of these possibilities involves drug delivery in many forms. The barriers protecting the eye hamper ocular drug delivery. Fungal keratitis is a serious and painful corneal disease caused by fungal organism. In India, there are approximately 6.8 million people who have corneal blindness and about a million have bilateral corneal blindness. Ocular diseases require localized administration of drug to the ocular tissues. The existing ocular drug delivery systems are fairly primitive, insufficient, and suffering with major drawback as a low ocular bioavailability. Here the article overview various aspects from development to some important evaluation parameters of a novel intracorneal drug delivery system for the management of fungal keratitis.

Keywords: Intracorneal drug delivery system, Fungal Keratitis, Corneal epithelium, Ocular bioavailability,

INTRODUCTION

"VISION 2020, THE RIGHT TO SIGHT", was the global initiative launched in the year 1999. According to the World Health Organization, corneal diseases are a major cause of vision loss and blindness, second only to cataract in overall importance. It is estimated that, worldwide approximately 180 million people are visually impaired; of these between 40 and 45 million are blind. Even more compelling, it is estimated that, worldwide approximately 180 million people are visually impaired; of these between 40 and 45 million are blind. Even more compelling, it is estimated that, worldwide approximately 180 million people are visually impaired; of these between 40 and 45 million are blind. Even more compelling, it is estimated that, worldwide approximately 180 million people are visually impaired; of these between 40 and 45 million are blind. Even more compelling, it is estimated that, worldwide approximately 180 million people are visually impaired; of these between 40 and 45 million are blind. Even more compelling, it is estimated that, worldwide approximately 180 million people are visually impaired; of these between 40 and 45 million are blind. Even more compelling, it is estimated that, worldwide approximately 180 million people are visually impaired; of these between 40 and 45 million are blind. Even more compelling, it is estimated that, worldwide approximately 180 million people are visually impaired; of these between 40 and 45 million are blind. Even more compelling, it is estimated that, worldwide approximately 180 million people are visually impaired; of these between 40 and 45 million are blind.

Gross anatomy and physiology of the eye

The physiological structure of the eye is shown in Fig. 1. The eye is housed in an eye socket, or orbit, within the skull. The orbit exceeds in size the soft tissue eyeball, or globe, by a considerable margin. The space between the globe and the orbit is "filled by fat and lined with a sheet of connective tissue known as the fascia bulbi, or Tenon's capsule. The function of Tenon's capsule is to provide a smooth socket permitting the free movement of the globe. Linkage of the globe to the central nervous system is achieved via the optic nerve, which together with the optic muscles and ophthalmic vasculature, traverses the orbital fat to reach the globe. When cosmetic replacement of the entire globe is undertaken, a cosmetic orbital implant is usually introduced.

Fig. 1: The structure of the eye showing the essential physiological features

The cul-de-sac normally holds 7-9µl of tears but can retain up to approximately 20-30µl without overflowing. The normal tear flow rate and film thickness are 1µl/min and 4-9µm. The normal pH of the tears is ~ 6.5-7.6. The drainage of instilled solutions (25-50µl) away from the front of the eye is essentially completed at around 90 sec.

Cornea

This outer layer is a collagenous protective tissue, which is slightly elastic. The posterior region of the layer is opaque and forms the sclera proper. The anterior part of the scleral layer is visible at the surface of the globe and forms the well known & white of the eye. The foremost portion of the anterior sclera forms a specialized transparent window known as the cornea (through which eye colour and the dark pinpoint of the pupil can be seen in living subjects). The function of the cornea is to admit light to the interior of the globe. Such light is refracted by passage through the cornea prior to its entry into the depths of the globe via the pupil. It has a radius of 7.6mm. It is approximately 0.5mm in central region and increasing to approximately 0.7mm at the periphery. The cornea is an auricular tissue to which nutrients and oxygen are supplied via bathing with lachrymal fluid and aqueous humor as well as from blood vessels at corneoscleral junction. Cornea composed of five layers, corneal epithelium, anterior limiting lamina, substantia propria or corneal stroma, posterior limiting lamina (Descement's membrane) and corneal endothelium.

Fig. 2: Cross-section of cornea

Corneal epithelium: main barrier

The corneal epithelium is ~50µm thick and represents most important barrier for the drug. The corneal epithelium, which has low porosity and high tortuosity due to tight annular junctions, is the main barrier for Hydrophilic drug, whereas the middle stromal layer which mainly of water interspersed with collagen fibrils and accounts for the most of the cornea's thickness is the main barrier for lipophilic drugs. This results not only in a low net eye drug
delivery, but also in substantial systemic availability of ocular drug after topical application, which also results in systemic side effect.6,7

Fungal keratitis
Fungal keratitis is a serious and painful corneal disease caused by fungal organism. Infection is exogenous, entering through the corneal epithelium. The insult of the cornea is usually a minor trauma, but it is occasionally due to other conditions or diseases that damage the epithelium. The common filamentous fungal genera involved in mycotic keratitis are Fusarium and Aspergillus spp. fungal keratitis may appear as a grayish-white lesion with feathery borders.8

Medical therapy
Currently, the therapy of fungal disease of the eye is unsatisfactory. The antifungal agents available today are mostly fungistatic, requiring a prolonged course of therapy. Although models of Aspergillus and Candida have been established, there are no reliable animal models of Fusarium keratitis. Fungi considered ocular pathogens are rarely encountered among the systemic mycoses. Thus, the therapeutic principles valid for systemic fungal infections may not apply to the cornea.9

Ocular drug delivery system
Topical ocular drugs are generally administered in the form of eye drops. The bioavailability of drugs administered as eye drops is severely limited by physiological constraints such as tear turnover and the blinking reflex. Further, drug loss due to naso-lacrimal drainage, conjunctival absorption and protein binding again results in poor bioavailability and systemic side effects. Consequently, frequent instillation of eye drops is required, resulting in pulsed administration and patient non-compliance.10,11 Gually, the main prerequisite for absorption of drugs into the eye is good corneal penetration and prolonged contact time with the corneal epithelium.

In any drug treatment, the overall goal of drug delivery is to achieve and maintain therapeutic concentrations of the drug at its site of action for sufficient time to produce a beneficial effect. Corneal absorption is much slower process than elimination for many drugs k loss (first order elimination rate) is approximately 0.5‐0.7/min and ka (first order absorption rate) is about 0.001/min. Therefore, the ocular bioavailability can be increased by decreasing k loss or by increasing k absorption and this can be achieved by modifying ocular dosage form having prolonged contact time with the corneal surface and better penetration through cornea.12 To enhance the transcorneal penetration of drug, its ocular bioavailability and at the same time to avoid/minimize the unwanted side effect resulted from frequent dosing a novel concept of intracorneal drug delivery system has been evolved.

Types of ophthalmic dosage forms
Available ophthalmic dosage forms are ophthalmic solutions, gel-forming solutions, powders for solutions, ophthalmic suspensions, ointments, emulsions, gels and ocular inserts.13 Available ocular dosage forms for the management of fungal keratitis are ophthalmic suspension of drug Natamycin and liposomal preparation of drug Amphotericin-B.

Ocular insert: background review
The first solid medication (precursors of present insoluble inserts) was used in 19th century, which consisted of squares of dry filter papers previously impregnated with dry solution (e.g. Atropine sulphate, Pilocarpine hydrochloride). Small section were cut and applied under eyelid.

Later, Lamellae, the precursors of present insoluble inserts, were developed. They consisted of glycerinated gelatin containing different ophthalmic drugs. Glycerinated gelatin "Lamellae" were present in official compendia until the first half of the present century.14 However, the use of lamellae ended when more stringent requirements for sterility of ophthalmic preparation were enforced.
Intracorneal drug delivery system: a novel approach

Development of dosage form or delivery system for the drug applied topically into the eye requires a deep understanding of anatomical and physiological characteristics of eye that offers a resistance to the foreign substances. The success of delivery system depends upon the duration to which this resistance can be compromised without affecting other tissues. From the last decades, an extensive research is going on to enhance the transcorneal penetration of drug for the better management of ocular disorders.

Most ocular treatments like eye drops and suspensions call for the topical administration of ophthalmically active drugs to the tissues around the ocular cavity. These dosage forms are easy to instill but suffer from the inherent drawback that the majority of the medication they contain is immediately diluted in the tear film as soon as the eye drop solution is instilled into the cul-de-sac and is rapidly drained away from the precorneal cavity by constant tear flow and lacrimo-nasal drainage. Therefore, the target tissue absorbs a very small fraction of the instilled dose and frequent dosing is required that has its own side effects. 27,28,29

Fungal keratitis not only infects the cornea but it starts multiplying in cornea and spread over other ocular tissues. To maintain the therapeutic concentration of drug in ocular tissue for the prolonged period, intracorneal drug delivery system can be used as a better strategy for the management of ocular disorder such as fungal keratitis.

The outer most layer of cornea i.e. corneal epithelium act as a main barrier in the absorption of the drug applied topically. Topically applied ocular drug have to reach the inner part of the eye and transcorneal penetration is believed to be the major route for drug absorption. To avoid all the hurdles to the drug absorption that are present at precorneal area (protein binding, drug metabolism and enzyme activity, nasolacrimal drainage, absorption through conjunctiva and tear flow) that reduces the ocular bioavailability of drug, the drug delivery system can be placed at stromal layer of cornea.

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**Table 1: Sort of ocular inserts**

<table>
<thead>
<tr>
<th>X</th>
<th>Name</th>
<th>Reported by</th>
<th>Year</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ocuser</td>
<td>Quigley et al. and</td>
<td>1975</td>
<td>Flat, flexible elliptical insoluble devise consist of two layers enclosing</td>
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<tr>
<td></td>
<td></td>
<td>Unguhati et al.</td>
<td>1980</td>
<td>a reservoir, used commercially to deliver Pilocarpine for 7 days.</td>
</tr>
<tr>
<td>2.</td>
<td>SODI</td>
<td>Khromov et al.</td>
<td>1976</td>
<td>Small oval wafer, composed of soluble copolymer consisting of acrylicide,</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N- vinyl pyrrolidine and ethyl acrylate, soften on insertion.</td>
</tr>
<tr>
<td>3.</td>
<td>Collagen shields</td>
<td>Bloomfield et al.</td>
<td>1977</td>
<td>Use of collagen inserts as a tear substitutes and as a delivery system for</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1978</td>
<td>Gentamicin.</td>
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<td>4.</td>
<td>Lacrisert</td>
<td>Lamberts et al.</td>
<td>1978</td>
<td>Rod shaped device made from Hydroxypropyl cellulose used for the treatment</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>of Dry eye syndrome.</td>
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<tr>
<td>5.</td>
<td>NODS</td>
<td>Lloyd et al.</td>
<td>1985</td>
<td>Medicated solid Polyvinyl alcohol flag that is attached to a paper covered</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>handle. On application, the flag detaches and gradually dissolves releasing</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>the drug.</td>
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<tr>
<td>6.</td>
<td>Mini disc</td>
<td>Bewa et al.</td>
<td>1985</td>
<td>4-5mm diameter contoured either hydrophilic or hydrophobic disc.</td>
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<td></td>
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<tr>
<td>7.</td>
<td>BODI</td>
<td>Gurtler et al.</td>
<td>1995</td>
<td>Adhesive rods based on mixtures of hydroxypropyl cellulose, ethyl cellulose,</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>poly acrylic acid and cellulose acetate phthalate.</td>
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<td>8.</td>
<td>Silicone rubber/ Hologen composite ophthalmic inserts</td>
<td>Chetoni et al.</td>
<td>1998</td>
<td>Cylindrical device containing mixtures of silicone elastomer and sodium</td>
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<td></td>
<td></td>
<td>chloride as a release modifier with a stable Polyacrylic acid (PAA) or</td>
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<td></td>
<td>polymethylacrylic acid (PMA) interpenetrating polymer network grafted on</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>to the surface.</td>
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<tr>
<td>9.</td>
<td>One-side-coated ocular insert</td>
<td>Sasaki et al.</td>
<td>2003</td>
<td>Prepared by attaching a polypropylene tape on the one side of the polymer</td>
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<td></td>
<td></td>
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<td></td>
<td>disc of poly (2-hydroxypropyl methacrylate) (HPM) containing tilsol as a</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>model ocularphic drug.</td>
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<td>10.</td>
<td>Molecularly imprinted soft contact lenses</td>
<td>Hiratani et al.</td>
<td>2004</td>
<td>Soft contact lenses consisted of N,N-diethylacrylamide, methacrylic acid</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>and ethylene glycol dimethacrylate. Timolol was used as a model drug.</td>
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<tr>
<td>11.</td>
<td>New ophthalmic mydriatic insert</td>
<td>Stephane et al.</td>
<td>2006</td>
<td>New insoluble-matrix retrolapbral opthalmic insert containing phenylephrine</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>and tropicamide.</td>
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<tr>
<td>12.</td>
<td>Ophtha Coil</td>
<td>Pijs et al.</td>
<td>2007</td>
<td>The ocular insert consists of a pradofloxacin-loaded adherent hydrogel on a</td>
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<td>thin wire, which is coiled. The inner lumen of coil was filled with a polymer</td>
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<td></td>
<td>rod made from a poly (2-hydroxyethyl methacrylate) hydrogel and loaded with</td>
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<td></td>
<td></td>
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<td></td>
<td>the same drug.</td>
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**Fig. 5:** Drug release and Ocular tissue distribution through a patch inserted in stromal layer of cornea.

**Mechanism of drug release from an implant**

Mechanism of controlled drug release from an intracorneal patch in to the eye is as follows. 22,23,29

**A. Diffusion**

In the diffusion mechanism, the drug is released continuously at a controlled rate through the membrane in to the fluid. If the insert is a solid non-erodible body with pores and dispersed drug. The release of the drug can be take place through the pores. In a soluble
device, true dissolution occurs mainly through polymer swelling. In swelling controlled device, the active agent is homogeneously dispersed in a glassy polymer. When the insert placed in to the stromal layer of cornea, water tends to penetrate the matrix, which follows the swelling process, polymer chain relaxation and drug diffusion takes place. Dissolution of matrix depends on the polymer structure, whether it is used as such or with some modification such as cross-linking which provides extra mechanical strength and controlled drug release. Release from these devices mostly found to be Fickian ‘square root of time’ kinetics. In some instances Non-Pickian, Zero order kinetics has been observed.

B. Bioerosion

In the bioerosion mechanism, the configuration of the body of the insert is constituted from a matrix of bioerodible material in which the drug is dispersed. Contact of the intracorneal insert with the fluid results in erosion of matrix and controlled release of the drug. The drug may be dispersed uniformly throughout the matrix but the controlled release of the drug is mostly occurred when the drug was superficially concentrated in the matrix.

Method

Intracorneal drug delivery system can be developed in the form of a small patch that can easily accommodate in the stromal layer of cornea. The patches are prepared by solvent casting method with help of suitable mould. After the patch developed, it can be cut into small pieces of suitable dimension that can easily placed in stromal layer of cornea.

Evaluation parameters

Estimation of drug content

Drug content in the patch is estimated by UV-Visible spectrophotometer. The patch was dissolved first in solvent in which polymer dissolved, so that the polymer get dissolved and kept for 1 hr under stirring then kept for 24 hrs. Similarly, a blank was carried out using drug free patch. Then absorbance will be measured at $A_{max}$ using UV-Visible spectrophotometer.

Surface pH

This will indicates that the prepared intracorneal insert would not alters the pH of the tear and will not cause any irritation after its application.

Patch thickness

Assessment of thickness can be done by using digital micrometer. The mean and standard deviation should be calculated.

Folding endurance

Determined by repeatedly folding one film at the same place till it broke. The number of times the film could be folded at the same place without breaking/cracking gave the value of folding endurance.

Loss on drying

Loss on drying is the loss in weight in % w/w resulting from water and volatile matter of any kind that can be derived off under specific condition. For each formulation 3 MCT where taken and weighed. Then the patch was kept in the incubator in open condition at a specific temperature. At different time intervals, the MCTs along with the patch were taken and weighed. This procedure was followed till concordant reading came.

Tensile strength

Tensile properties indicate how the material will react to forces being applied intensity. Tensile tests are used to determine the modulus of elasticity, elastic limit, elongation, proportional limit, tensile strength, yield point, yield strength and other tensile properties.


