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Thakur, R., Tekko, I., McAvoy, K., Donnelly, R. F., McMillian, H., & Jones, D. (2017). Minimally invasive microneedles for ocular drug delivery. *Expert Opinion on Drug Delivery*, *14*(4), 525-537. https://doi.org/10.1080/17425247.2016.1218460, https://doi.org/10.1080/17425247.2016.1218460

Published in:
Expert Opinion on Drug Delivery

Document Version:
Peer reviewed version

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Journal:	*Expert Opinion on Drug Delivery*
Manuscript ID	EODD-2016-0127.R1
Manuscript Type:	Review
Keywords:	Ocular drug delivery, Posterior segment, Anterior segment, Microneedle, Minimally-invasive

REVIEW

Minimally invasive microneedles for ocular drug delivery

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Abstract

Introduction: Anterior and posterior segment eye diseases are highly challenging to treat, due to the barrier properties and relative inaccessibility of the ocular tissues. Topical eye drops and systemically delivered treatments result in low bioavailability. Alternatively, direct injection of medication into the ocular tissues is clinically employed to overcome the barrier properties, but injections cause significant tissue damage and are associated with a number of untoward side effects and poor patient compliance. Microneedles (MNs) has been recently introduced as a minimally invasive means for localizing drug formulation within the target ocular tissues with greater precision and accuracy than the hypodermic needles.

Areas covered: This review article seeks to provide an overview of a range of challenges that are often faced to achieve efficient ocular drug levels within targeted tissue(s) of the eye. It also describes the problems encountered using conventional hypodermic needle-based ocular injections for anterior and posterior segment drug delivery. It discusses research carried out in the field of MNs, to date.

Expert opinion: MNs can aid in localization of drug delivery systems within the selected ocular tissue. And, hold the potential to revolutionize the way drug formulations are administered to the eye. However, the current limitations and challenges of MNs application warrant further research in this field to enable its widespread clinical application.

Keywords: Ocular drug delivery, Posterior segment, Anterior segment, Microneedle, Minimally-invasive

Article highlights box

- Visual impairment and blindness are potentially the most devastating health problem worldwide.
- Drug delivery to the eye is challenging due to the extremely delicate nature, relative inaccessibility, and barrier properties of ocular tissues
- Topical and systemic routes of ocular drug delivery result in low or sub-therapeutic drug levels; drug delivery implants need surgical implantation.
- Injections into the eye using conventional hypodermic can provide direct access to the target tissues. However, this method is highly invasive and causes considerable discomfort, pain and associated with a number of side effects
- Microneedles (MNs) could offer minimally-invasive means of ocular drug delivery, less tissue trauma, less drug dosage and precise localisation of the medication.
- MNs allow precise injections within the thin ocular tissues (e.g. sclera and cornea) – an advantage for localized drug delivery
- MNs when integrated with sustain drug delivery formulations can offer long-term localised drug delivery in treating both anterior and posterior segment eye diseases.

1. Introduction

Visual impairment and blindness are potentially the most devastating health problem worldwide. The World Health Organization (WHO) estimates that globally about 285 million people are visually impaired of which 39 million are blind, and 246 have a low vision [1]. Ocular diseases can be broadly classified into anterior and posterior segment diseases. Anterior segment diseases that can cause serious vision impairment or discomfort include corneal neovascularization (CNZ), glaucoma, bacterial/fungal keratitis, uveitis, herpes simplex keratitis, blepharitis and dry eye syndrome. Additionally, diseases that originate in the posterior segment of the eye lead to permanent loss of vision, if left untreated, and account for the majority of blindness, such as in age-related macular degeneration (AMD), diabetic retinopathy, diabetic macular edema, cytomegalovirus retinitis, and other chorioretinal diseases [2].

In general, conditions that affect the anterior chamber are less likely to be sight threatening compared to those that affect the posterior segment. Nevertheless, drug delivery to the eye can be challenging, owing to the extremely delicate nature of the ocular tissues concerned, their relative inaccessibility, and barrier properties of ocular tissues [3,4], which hinders efficient drug diffusion to target tissues. For example, posterior segment of the eye, which includes the retina, choroid, and vitreous body, is difficult to access due to the recessed location within the orbital cavity.

To date, multiple approaches have been used to deliver drugs to the eye such as systemic, topical, periocular (or transscleral) and intravitreal routes. Topical (e.g. eye drops) and systemic (e.g. oral tablets) routes result in low or sub-therapeutic drug levels due to multiple ocular barriers, requiring administration of unnecessarily high concentrations of drug that causes drug-related toxicity and producing low treatment efficacy [5]. To overcome the barrier function of the eye and to enhance localization of the drug close to the target tissues, injections are given either directly into the eye (intravitreal injection, IVT), around the outer surface of the eye (periocular or transscleral route) or within the tissues (intracorneal and intrascleral). These injections are given using conventional hypodermic needles. Although periocular route is considered to be less invasive than the IVT, transient diffusion of a drug across the sclera is limited. Drug diffusion across the scleral membrane is dependent upon drug's solubility, molecular weight/molecular radius, charge and polarity [6].

However, this method has shown low intraocular bioavailability due to a delay in diffusion through the sclera, systemic clearance and loss of drug before reaching the target tissues (e.g. retina) [7]. One of the standard treatments to overcome limitations of periocular injections is either an IVT, for posterior segment diseases, or intracorneal injections, for anterior segment diseases.

Using conventional hypodermic needles for intraocular injections is known to causes considerable discomfort, pain and requires a specialized set of skills. Notably, traditional injections given on frequent basis and over long-term may increase the chances of severe ocular complications and poor patient compliance. Therefore, there is a high demand for less invasive technologies that not only enhance patient compliance but also allow localised and precise drug delivery to the eye. In this regard, application of minimally-invasive microneedles (MNs) for ocular drug delivery is a relatively new concept. To date, only limited work has been done in this area. Therefore, this review article seeks to provide an overview of - typical challenges that are often faced to achieve efficient ocular drug levels within targeted tissue(s) of the eye; problems encountered using conventional hypodermic needle-based ocular injections; and how minimally-invasive MNs could assist in overcoming these challenges in treating sight-threatening eye diseases. It also provides an overview of the limitations and difficulties of MNs application to the eye and its prospects. Furthermore, to the author's knowledge, this is the first review of MNs application for ocular drug delivery, which is aimed to benefit researchers in this field.

2. Challenges and Obstacles of Ocular Drug Delivery

Although eye offers a convenient site for drug administration for various conditions, there are many challenges. Drug delivery research has significantly increased for other routes such as oral and transdermal routes, whereas progress in the area of ocular drug delivery has been gradual and relatively limited. Lee and Robinson in 1986 described the majority of ocular drug delivery systems as 'primitive and inefficient' [9], referring mainly to solutions, suspensions, and ointments. In 1995 around 90% of the ophthalmic formulations on the market were based on these three systems [10]. This statement can still be employed to describe a large number of systems currently used for the treatment of ocular conditions, although substantial advances have been made to enable targeting of ocular tissues in recent years, and

more sophisticated treatment strategies are currently under development [5, 11]. Many researchers attest to the difficulties of effective and efficient drug delivery to the eye, primarily due to the range of ocular barriers that are crucial in maintaining healthy physiological function but pose a variety of challenges for drug delivery. Following sections briefly, discuss the challenges faced and need for invasive procedures to overcome the barrier function of ocular tissues.

2.1 Anterior Barriers

In the anterior segment of the eye, the first challenge to drug delivery is the precorneal lacrimal fluid. Lacrimal fluid turnover and clearance is approx. 1 μL/min [12] *via* the nasolacrimal duct. Therefore, formulations instilled to the eye are cleared from the ocular surface in a matter of minutes [13]. Additionally, the lacrimal fluid is rich in peptides and proteins, which are capable of binding drug molecules and inhibiting their release or permeation [14].

The next barrier encountered in the anterior segment is the cornea. The cornea is the clear, outer layer of the eyeball, with dual action of limiting the entry of exogenous substances into the eye and protecting the ocular tissue. This tissue in an adult human has an average dimension of 11.5 mm horizontally, 10.5 mm vertically with an mean surface area of 1.3 cm^2, representing around 7% of the total surface area of an eyeball. The thickness in the central region is around 0.52 mm and increases towards the periphery [15]. Cornea is a multi-layered tissue composed of five distinct layers; from anterior to posterior they are the epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium, which affect the transport of drug molecules into the eye. The epithelium layer with an approximately of 50 μm in thickness consists of 5-7 layers of superficial, wing and basal epithelial cells. This layer forms a significant barrier to topical ophthalmic formulations, especially for hydrophilic and macromolecular drugs due to the barriers lipoidal nature and the tight junctions between the cells, importantly in the superficial epithelium cells [17,18]. Drug molecules require a partition coefficient of greater than 1 to adequately permeate the epithelium [19]. The molecular weight of hydrophilic molecules also plays a major factor in their permeation through the corneal epithelium [20] with those larger than 60-100 Da being unable to pass [20, 21].

The stroma is the second layer and accounts for >90% of the cornea thickness with an approximate thickness of 500 µm. It mainly consists of an extracellular matrix, stromal cells, and approximately 4% glycosaminoglycans [22]. Water-soluble molecules readily traverse this layer, and even high molecular weight drugs diffuse with ease [17, 23]. However, it restricts the movement of lipophilic drugs and macromolecules with a molecular weight of > 50,000 Da [24]. The endothelium layer consists of a monolayer of cuboidal cells with an approximate thickness of 5 µm [16]. Both the epithelium and the endothelium are hydrophobic in nature, providing a barrier to the movement of hydrophilic molecules across the cornea. However, the endothelium is approximately 2.7 times more permeable than the epithelium [25].

The thin, semi-transparent mucous membrane of the conjunctiva provides another challenge to anterior drug delivery. The vast presence of localised blood capillaries and rich lymphatic system within the conjunctiva, result in the rapid clearance of drug molecules. This significant drug loss into the systemic circulation has the issue of not only lowering the ocular bioavailability but can lead to unwanted systemic exposure of the drug [26].

2.2 Posterior Barriers

The posterior segment of the eye contains its own array of barriers such as sclera, choroid, and blood-retinal barrier (BRB), resulting in numerous challenges to drug delivery.

Scleral tissue offers mechanical support and strength to the eye. It covers approximately 80% of the eyeball surface and forms relatively a large surface area 16.3 cm^2 [27]. It is an elastic, tough, vascular, opaque white-yellow and microporous tissue composed of collagen and elastin fibres entwined with proteoglycans [28]. Scleral thickness varies throughout its circumference. In humans, the mean scleral thickness is reported to be 0.53 mm, with the thickness portion being approximately 1 mm at the posterior, near the optic nerve, and the thinnest portion being 0.39 mm at the equator [27]. Sclera consists of four layers they are from outer side to the inner side: Tenon`s capsule, episclera, stroma and lamina fusca [15]. Besides not having epithelium and endothelium layers, the scleral tissue differs primarily from the corneal tissue in the uniformity of the arrangement of the collagen fibres and the degree of hydration [29]. Relative to the cornea, the sclera has irregular collagen

fibres and a 4-fold lower concentration of proteoglycans resulting in lower water content, i.e., 68% in comparison to 78% in the corneal stroma [29]. Besides, the sclera is perforated by blood vessels and has an extensive nerve supply [29]. Due to the sclera's high aqueous content, hydrophilic molecules can diffuse through this layer more readily than hydrophobic molecules. The sclera is permeable to high molecular weight compounds and even proteins of 150 kDa [30], however, permeability declines exponentially with increase in the molecular radius [31]. The charge of the drug molecule also presents a challenge to penetration through the sclera; for example, positively charged molecules are at risk of interacting with the negatively charged proteoglycans within the sclera [28].

The choroid is one of the most highly vascularised regions of the body, and its primary function is to supply blood, rich in oxygen and nutrients, to the retina [32]. Bruch's membrane, located between the choroid and the retinal pigment epithelium (RPE), also functions as a barrier to the movement of vessels from the choroid into the RPE and retina. With increased aging the choroid has been shown to thin [33,34]. In contrast, Bruch's membrane thickens with increasing age, causing a disruption of its barrier activity, giving rise to some ocular diseases [35]. Changes in thickness within the choroid and Bruch's membrane can affect successful drug permeation and penetration from subconjunctiva and sclera, resulting in decreased drug delivery to the retina [32].

The BRB acts to restrict entry of unwanted molecules from choroid into the retina. It is the most significant barrier to systemic drug delivery. Following systemic administration drug molecules can enter the highly vascularized choroid relatively easily, but are commonly unable to pass the BRB. The BRB is extremely efficient in performing this restricting function due to its unique composition. The outer portion is formed by the retinal pigment epithelium (RPE), and the inner portion of the barrier is formed by the tight junctions of retinal capillary endothelial cells [13,36].

The retina is the intended site of action for most drugs delivered to the posterior segment of the eye. It does not have its own barrier function but can present challenges to drug delivery. The inner limiting membrane, which separates the retina and the vitreous humour, is composed of 10 distinct extracellular matrix proteins and is thought to prevent the penetration of some drug molecules into the retina [32].

However, it has been shown that anti-vascular endothelial growth factors (VEGF) such as bevacizumab (Avastin®, Genentech Inc.) with a molecular weight of 149 kDa, can successfully penetrate into the RPE *via* IVT route [37].

3. Ocular injections to overcome barrier functions

Regarding ocular drug delivery, the choice of route of administration or type of delivery system is very much dictated by the target tissue and potential barriers that need to overcome. Table 1 summarises different routes of ocular drug delivery along with their benefits and challenges. As can be appreciated from the information provided in Table 1, each route and method of administration have its advantages and disadvantages. However, this review is primarily focused on minimally-invasive means of ocular drug delivery using MNs. Hence, we will discuss challenges that are faced using highly-invasive conventional hypodermic injections in delivering drugs to the eye (Fig. 1). Hypodermic needle-based injections are clinically employed to gain direct access to the target tissues to overcome barrier function of the eye, in treating a number of diseases.

3.1 Anterior segment injections

Topical administration of eye drops has very low ocular bioavailability (< 5%). Therefore, frequent drops are necessary, yet it is only effective in treating diseases of the front of the eye. Whereas, due to biological barriers, the systemic administration has to be given at very high doses which cause systemic toxicity.

Therapies used to treat diseases of the anterior segment of the eye have been widely researched and are well documented. Formulation approaches for treating anterior eye diseases include eye drops, gels, suspensions, and emulsions, to name a few. However, the most commonly formulated preparation is topical eye drops that have the advantage of being non-invasive and can be easily self-administered, resulting in good patient compliance. Nevertheless, topical administration is inefficient due to the barrier properties of corneal epithelium, thus requiring either frequent administration of medication or high doses – especially in treating certain corneal conditions such as CNZ**,** dystrophy, fungal and bacterial keratitis, which may lead to vision impairment or loss if not treated effectively [38]. As a result, direct injections of medication are commonly practised in treating these conditions such as subconjunctival, intrastromal,

intracameral, or intracorneal injections (Fig. 1). These injections enable achieving high drug concentrations within the specific tissue of the anterior segment of the eye, and found to be particularly beneficial in the emergency management of acute conditions (e.g. CNZ and fungal keratitis).

In subconjunctival injections, selected medication is directly delivered in subconjunctival space (Fig. 1). It is considered to be most patient friendly than any other types of ocular injections. Most commonly hypodermic needles of sizes ranging from 21-30G are used for subconjunctival injections, with injections volumes of up to 0.1 ml. For example, in treating CNZ, bevacizumab (Avastin®) was administered by topical route, at a concentration of 2.5 mg/ml eye drop (10 µL) given 5-times per day [39], but higher concentration (4, 5 or 10 mg/ ml) eye drops were given only 2-times per day. Here, a frequent administration is required due to poor penetration of the bevacizumab, which is a high molecular weight (149kDa) hydrophilic drug. Alternatively, to lower drug concentrations and reduce the frequency of administration, subconjunctival injections were given at 1.25 mg or 2.5mg/0.1 ml with lower frequencies [40-42]; this demonstrates advantages of injections over topical delivery. Although subconjunctival injections guarantee better delivery than topical eye drops, local side effects – such as hemorrhage, have been reported [43]. Additionally, rapid drug elimination following subconjunctival administration is also well documented, which results in drainage of formulation into systemic circulation thereby lowering ocular bioavailability [44]. The short residence time limits the effective permeation of drug molecules through multiple ocular barriers before reaching their intended site of action at either back or front of the eye.

Alternatively, intrastromal, intracameral, or intracorneal injections allow direct administration of the medication within the target tissue. For example, intrastromal injections (Fig. 2) have been widely used as a mean of effecient drug delivery especially in the management of CNZ [43,45] and fungal keratitis [43,46]. For example, using a 31G needle intrastromal injection of bevacizumab, approx. 10 µL (100 µg), was performed in human eyes. In certain cases, multiple intrastromal injections were given in the same eye [43], so as to accommodate a higher amount of drug per eye. In another study, patients who were unresponsive to topical antifungal therapy, targeted delivery of voriconazole was achieved by intrastromal injections (50 µg/0.1 mL using 30G needle), which was found to be effective to treat deep

recalcitrant fungal keratitis. Five divided doses (i.e., five intrastromal injections) were given around the infiltrate to form a deposit of the drug around the circumference of the lesion to ensure the formation of a barrage of intrastromal voriconazole around the entire infiltrate, to enhance the efficacy of voriconazole [47]. Although, intrastromal injection using a hypodermic needle have shown promising results [45,46], it is unpleasant for patients; it is associated with series of ocular complications and side effects including being painful and highly-invasive; possibility of imposing bacterial infections; inflammation and tissue damage; and requires expertise in clinical administration [48,49]. More importantly, delivering precise volumes of drug solutions/suspensions, often < 10-25 μL, within the thin corneal tissue of 0.52 mm thickness is technically challenging and highly impossible to produce reproducible results in each patient. Thus, varying dosages will lead to different levels of therapeutic efficacy among the patients.

3.2 Posterior segment Injections

Delivery of drug molecules, to treat visually impairing ocular conditions that originate in the posterior segment of the eye, has been the most challenging task to the pharmaceutical scientists and retinal specialists. Patient-friendly administration routes such as oral and topical dosage forms provide ineffective drug delivery to the posterior segment; thus direct injections in the eye, IVTs (Fig. 1), were found to be effective. In fact, IVTs have become the 'gold standard' to allow localised delivery of drugs to the back of the eye, with millions of injections given each year for patients suffering from a range of eye diseases worldwide.

IVTs were first utilised in 1911 to introduce air into the eye to repair retinal detachment [51]. Since then, their use has evolved as a method of repairing ocular ailments and delivering a range of therapeutics for the treatment of numerous ocular conditions, especially those of the posterior segment. Over the last number of decades, the use of IVTs has risen considerably; with these injections being one of the most frequently performed medical procedures in the US [52]. It is also estimated that in the UK in a department with around 500,000 patients in their care, 50-100 of these injections are performed weekly [53]. IVTs allow localised delivery of therapeutics and therefore reducing any systemic adverse effects [54]. According to the Royal College of Ophthalmologists guidelines on IVTs, the needles used should be 30G

needles non-colloidal clear solutions and 27G for particulate preparations. The injection needle length should be 12 to 15mm i.e. 1/2 to 5/8 inch, with a maximum injection volume of 100 μL [55].

Although IVTs are not overly patient-friendly, they are capable of overcoming multiple ocular barriers and deliver adequate drug concentrations almost directly to the site of action [56]. Nevertheless, IVT being invasive method is associated with multiple adverse effects and complications – e.g. raised intraocular pressure (IOP), discomfort or pain (despite the use of anesthesia), intraocular inflammation, retinal detachment, haemorrhage, endophthalmitis, cataract, lens damage and potentially blindness [4,57,58]. All of these issues require supplementary medication. In treating chronic ocular diseases such as AMD, repeated injections, every 4-6 weeks, are required, indefinitely. Frequent injections will significantly increase the burden on patients and physicians. Furthermore, intravitreal delivery with conventional hypodermic needles should strictly adhere to numerous safeguards to avoid mechanical injury to the lens and retina [59]. These risks are dependent upon the needle type, where lower gauge needles cause more pain and higher damage to the eye. Therefore, smaller needles, 27 to 30 G, are preferable.

Drug formulations can also be injected on the outer surface of the eyeball, through periocular injections (transscleral delivery) such as sub-tenon, retrobulbar, peribulbar and posterior juxtascleral (Fig. 1), which are considered to be less invasive than IVT. Transscleral delivery *via* periocular administration is thought to be one of the safest means of achieving stable drug concentrations within the vitreous and retina, although there have been reports of anterior segment complications after periocular injection such as raised IOP, cataract, and strabismus. Other challenges to drug delivery *via* the transscleral are dependent on the nature of the drug molecule. Interestingly, the sclera is highly permeable to large drug molecules; however the RPE is a significant barrier to diffusion for both these macromolecules and hydrophilic drug molecules, it may be the rate-limiting feature in the delivery of these molecules *via* the transsceleal route to the retina [60]. While molecular weight isn't a major factor in drug delivery *via* the transscleral route, molecular radius of the drug molecule is. It has been shown that a smaller molecular radius will result in increased permeability through the scleral tissue [30].

Conventional hypodermic injections are capable of delivering drug formulations to the target site, but numerous adverse effects and risks associated with conventional injections are still a major problem. Although tremendous research interest in developing novel sustained release formulation is ongoing, so as to maintain constant drug levels at the target ocular tissues for prolonged periods and reduce the frequency of injections – technologies that enable safe delivery of the existing or new sustained release formulations are still limited. Therefore, in an attempt to overcome highly invasive ocular injections using standard hypodermic needles, and safer delivery of medication minimally-invasive MNs devices was found to be of significant interest.

4. Minimally-invasive MNs for ocular drug delivery

MN is an attractive technology that offers minimally-invasive drug delivery. MNs have been extensively investigated over the last 15 years to enhance transdermal drug delivery and therapeutic drug monitoring [62-64]. MNs are typically 25–2000 μm in height and have been fabricated from a wide range of materials and in different shapes. For further information, readers can refer to our MN book for details about methods of MN fabrication and its application in transdermal drug delivery [65]. The materials that have been most commonly used in the fabrication of MNs are silicon, steel, glass or polymer to form either solid and hollow type MNs. The painless application of MNs has significantly increased research interest in the MNs application for drug delivery, therapeutic monitoring and cosmeceutical applications. Consequently, benefits of MN application to the eye could offer several advantages over invasive intraocular injections that utilize long conventional hypodermic needles. The MNs are long enough to overcome the ocular barriers with potential advantages including – bypassing ocular barrier function (e.g. epithelium and sclera); allowing localised delivery of drug molecules within the ocular tissue (e.g. intrascleral and intrastromal delivery); minimizing pain, tissue damage and reduce the risk of infection; increase patient compliance due to nearly invisible needles, and the potential of providing a localized drug depot to achieve target drug delivery to the eye.

In general, the transdermal application of MNs can be achieved *via* one of the following strategies in order to deliver therapeutics [66] (Fig. 3):

- A 'poke with patch' strategy that involves the application of a solid MN arrays to create micropores and further removal of arrays followed by the administration of a drug formulation – as a patch, a gel or a solution. Movement of molecules through microchannels occurs *via* passive diffusion thereby providing enhanced drug delivery.

- A 'coat and poke' strategy that relies on coating a drug formulation onto the MNs and subsequent insertion of the coated MN array into the tissue. The drug is deposited within the tissue by the dissolution of the coating.

- The third mode of drug delivery *via* MNs utilizes incorporation of drug molecules into the structure of polymeric MNs and subsequent insertion into the skin. The drug delivery depends on the rate of polymer dissolution or degradation within the skin.

- Drug molecules can also be transported across the tissue *via* injection through hollow MNs, which is similar to the application of hypodermic needles [67].

- Swelling MNs fabricated using polymers have been developed more recently. Following insertion into the skin, MNs imbibe tissue fluid and allow drug diffusion from a drug reservoir through the swollen polymeric matrix of the MNs [62].

In reality, using MNs for drug delivery to the eye is a fairly new concept since very little research has been carried out in this field. To date, in enhancing ocular drug delivery using MNs, only three of the above five strategies of MN application have been investigated namely coated, soluble and hollow MNs. Primarily these three modes of MN application allow instant delivery and retrieval of the MNs (or its baseplate), which imitate the administration of conventional hypodermic needles to the eye. Literature indicates the use of either single solid or hollow MNs for ocular delivery of drug molecules of various molecular weights including sustain release nanoparticles, microparticles or depot forming gels – where the MNs were fabricated using silicon, stainless steel or glass.

Prausnitz and co-workers were first to demonstrate the application of coated MNs to the eye [49]. In this study, Jiang *et al.* 2007 reported drug delivery into the anterior

segment of the eye using coated MNs (Fig. 4a). Individual stainless steel MNs measuring 500-750 µm in length and 200 x 50 µm in width, and 55° in tip angle were tested for anterior and posterior drug delivery *via* either intrascleral or intracorneal routes, respectively. MN was coated with model drug sodium fluorescein (approx. 280 ng) and inserted halfway into the cornea of a rabbit eye and left in place for 2 mins and then retrieved back. After 1 min following MN insertion, a sharp increase of intraocular fluorescein concentration and then gradually further increase peaked at 3 hrs and then gradually decreased to background within 24 hrs. This study showed that the drug depot was formed within the cornea, which steadily released fluorescein into the anterior segment for hours. Although a small abrasion was noted at the site of MN insertion, it disappeared after 3 hrs. The study showed MN was able to achieve a 60-fold increase in fluorescein in comparison to topical application. In this study, experiments were also performed using pilocarpine-coated MN, which showed a 45-fold increase in its bioavailability relative to topical administration. Jiang *et al*. 2007 used the same individual stainless steel MN coated with model drugs i.e. sulforhodamine, protein, and DNA to be delivered to the posterior segment of the eye. The study revealed that MN penetrated in the human cadaver sclera to a depth of 300 µm. The drug coating rapidly dissolved off the needles within the scleral tissue within 20 sec after insertion.

In another study, Jiang *et al.* 2008 demonstrated intrascleral delivery using a hollow glass MN not only for a simple model drug (sulforhodamine), but also micro/nanoparticles formulations [68]. The MN was fabricated from a borosilicate cylindrical glass micropipette tubes with 1.5 mm outer diameter and 0.86 mm internal diameter (Fig. 4b). Needles were initially inserted into the tissue at a depth of 700-1080 µm, and retracted out of the tissue in increments of 60 µm during the solution injection. Sulforhodamine solution was then infused at a pressure of 15 psi. No solution was delivered into the tissue after the initial insertion. Upon further retraction from 200 to 300 µm, the delivery was achieved at volumes of 10 to 35 µL of fluids containing either soluble drug molecule sulforhodamine B or nanoparticles suspensions from an individual MN. However, microparticles were only delivered in the presence of hyaluronidase and collagenase spreading enzymes. The enzymes in this case were used to breakdown the tissue components so as to accommodate the microparticles.

Unlike intrastromal or intrascleral injection using MNs, Patel *et al.* 2011 [69] investigated posterior drug delivery in suprachoroidal space (SCS) using hollow MN. The SCS is a potential space between the sclera and choroid that goes circumferentially around the eye. Being immediately adjacent to the choroid and retina, delivery in SCS can offer targeted drug delivery to these tissues. As like above, a single glass hollow MN measuring 800-1000 µm in length were used to infuse nanoparticle and microparticle suspensions into the SCS in *ex vivo* rabbit, pig and human eyeballs. MNs were shown to deliver sulforhodamine B as well as nanoparticle and microparticle suspensions into the SCS of rabbit, pig, and human eyes. Volumes up to 35 µL were administered consistently. The study suggested that particles of 20 and 100 nm could spread within the sclera as well as the SCS, whereas particles of 500 and 1000 nm localised exclusively in the suprachoroidal space (Fig. 4c). To deliver 500 - 1000 nm particles in the SCS, a minimum MN length of 1000 µm and a pressure of 250–300 kPa were necessary. Similarly, Patel *et al.* 2012 [70] used metal MNs fabricated from 33G needle cannulas, with 750 µm in length and the bevel at the orifice, to evaluate ocular pharmacokinetics of different molecules (sodium fluorescein, fluorescein isothiocyanate dextrans of 40 kDa and 250 kDa, and bevacizumab tagged with Alexa-Fluor 488) and particles (FluoSpheres) injected into the SCS of the rabbit eye. Here, the metal MNs were attached to a 1-mL syringe. In general, the molecules were cleared from the SCS within 1 day; therefore, particles were injected into the SCS so that the drug can be localized and remain for months. Particles of 20 nm to 10 µm diameter were injected into the SCS of rabbit eyes, *in vivo,* which remained within the SCS and choroid for at least 2 months. It was noted that the capillary drainage might play a role in clearance from the SCS. Nevertheless, this study demonstrated the ability to localize particles with in the SCS for sustaining drug delivery.

In a recent *in vivo* study, Gilger et al. 2013 used the above 33G hollow MNs, 850 µm in height, to deliver triamcinolone acetonide (TA) to the SCS [71]. The study have demonstrated that 0.2 mg and 2.0 mg of the SCS TA was as effective in reducing inflammation as 2.0 mg of TA by IVT in a model of acute posterior uveitis inflammation. Furthermore, there was no evidence of adverse effect – i.e. increase in IOP, drug toxicity, or hemorrhage following MN application. Likewise, Chiang et al. 2016 recently have investigated the circumferential distribution of particles in the

SCS of rabbit and human cadaver eyes [72]. Same hollow MNs were used as reported by [69] i.e. a 33G needle with 750μm height. A 200 nm diameter red-fluorescent microspheres with injection volumes ranging from 50 - 200 μL were performed in the SCS. In rabbit eyes, particles when injected in the superior or inferior hemispheres did not significantly cross into the other hemisphere, due to a barrier formed by the long posterior ciliary artery. In human eyes, the short posterior ciliary arteries prevented circumferential spread towards the macula and optic nerve. Therefore, suggesting that the anatomical barriers could hinder even spread of the administered drug or formulation within the SCS. Therefore the judicious selection of a region for injection is essential.

Kim *et al.* 2014 [60] investigated using single solid stainless steel MN measuring 400 μm in length coated with bevacizumab to treat CNZ. Results revealed that drug was delivered intrastromally and allowed dramatic dose sparing compared with subconjunctival and topical eye drops – providing just 4.4 μg of the drug needed to produce similar effect as much as 2,500 μg *via* subconjunctival injection and 52,500 μg when delivered *via* eye drops.

Song *et al.* 2015 [73] designed MN-based pen type device (Fig. 4d) to enhance the reliability of MN insertion, so as to allow easy insertion into a small target region of ocular tissue. A solid SU-8 resin based MN was fabricated and attached to a macro-scale applicator to create the MN pen. The resulting MN had the base area of 200 × 200 μm^2 with the height of 140 μm. Rhodamine B, evans blue or sunitinib malate was used, along with polymer carrier, as a model drugs to dip coat the MN. It was shown that the MN pen enabled precise localization of drug within the stromal membrane of cornea, which is otherwise difficult to achieve when given topically due to corneal epithelium.

Matthaei et al. 2012 [74], to improve reproducibility of injection method using hand-held syringes, compared different type of hollow MNs and syringes and quantified the intrastromal distribution of Indian ink in mouse cornea by injections of different volumes (1 and 2 μL). Needles types and syringes tested were namely 33 G (attached to a 2.5 μL syringe), 35 G needles (attached to a 10 μL syringe) and glass MNs beveled to 25° and an inner tip diameter of approximately 50 μm (attached to a 2.5 μL syringe), respectively. Injections of 1 μL and 2 μL resulted in an overall mean of 49%

and 73% respectively of total corneal area involved. The use of 33 G metal needles provided the most reliable and effective outcomes, whereas the glass MN tips broke within the stroma in 25% of cases which is undesirable and create potential safety concerns. Irrespective of needle type, a small amount of leakage was noted in all cases [74].

Unlike the single HMNs or coated MNs, Palakurthi et al. 2011 [74] investigated MNs that were fabricated into an array of 3x3 biodegradable methotrexate loaded MNs with 2 mm in length, 2 mm in width, and 2.3 mm in height. The MNs were surgically placed in the deep lamellar scleral pocket in rabbit eye, *in vivo*, were found to be safe. The fundamental advantage of using MNs is its ability for painless or minimally-invasive nature due to its micron-sized dimensions. However, in this study the term *microneedle* perhaps needs reconsideration, as the MNs were surgically implanted and were much higher in dimensions than those employed in both ocular and transdermal application.

Long-acting ocular drug delivery systems such as micro-/nano-particles, liposomes, *in situ* implant forming gels and preformed solid implants are gaining tremendous interest due to their ability in maintaining constant drug levels following single administration [75]. However, administration of these formulations by either using standard hypodermic needles or surgical implantation would still hamper patient compliance. For example, some studies have previously developed and evaluated the administration of sustained release preformed intrascleral implants [76-79], as show in Fig 5 a and b. Although these intrascleral implants showed sustained drug release, they necessitate surgical administration within the thin tissue of sclera, which would have concerns greater than that seen with IVT injections. Additionally, any surgical procedure will only impose further costs and technical challenges with the treatment modality. We have recently demonstrated minimally invasive means of administering implants within the scleral tissue using HMNs [67]. In this study, we have shown administration of *in situ* implant forming thermoresponsive poloxamer-based gels into the scleral tissue to provide sustained drug delivery. HMN devices of 400, 500 and 600 μm in height were fabricated from hypodermic needles (i.e. 27, 29 and 30 G) and tested for depth of penetration into rabbit sclera. We have seen sustained release of fluorescein sodium over 24 h which varied with the depth of gel delivery in the sclera.

In fact, upon HMN injection the gel turned into a semi-solid implant and effectively encapsulated within the sclera to form an intrascleral implant, as seen in the Fig. 5c. Such methods of implant formation, without the need for surgical intervention, would aid or enhance patient acceptability, and at the same time overcome a number of side effects that are commonly seen with surgical administration.

5. Conclusion

Ocular drug delivery is gaining significant interest among academia and pharmaceutical industry. However, the barrier function of the eye remains a significant challenge for successful anterior and posterior segment drug delivery. Currently, management of sight-threatening eye diseases requires frequent injections of medication either within the ocular tissues (intrastromal/intrascleral) or directly into the eyeball (IVT) using conventional hypodermic needles. Nevertheless, frequent administration of medication using hypodermic needles is associated with numerous side effects and has poor patient compliance. Therefore, application of minimally-invasive MNs could offer numerous advantages to overcome the current issues surrounding hypodermic injections, as demonstrated by a number of studies in the past few years, which are discussed in this review. The advancing nature of research into MN delivery systems shows continual improvement in the ocular delivery of therapeutics. Moreover, Clearside Biomedical Inc. has recently demonstrated advantages of ocular drug delivery, in the SCS, using hollow MNs, which is currently in clinical trials (Phase 1/2) [80]. Although at its early stage, a number of parameters in relation to MN application to the eye warrants further investigation; for example, optimum MN design; volumes of injections *vs.* forces of application; safety, precision, accuracy and reproducibility; and manufacturing costs. Finally, MNs has significant potential to offer combined benefit of being minimally-invasive in application and ability to provide sustained localised drug delivery, which will provide significant benefits in overcoming current challenges faced by frequent intraocular injections using hypodermic needles.

6. Expert Opinion

Ocular drug delivery is notoriously difficult and unfortunately many conditions of the eye, if not treated effectively, can cause visual impairment or blindness. Treating eye diseases is challenging, owing to the extremely delicate nature and recessed location of the ocular tissues. Conventional routes such as topical eye drops or systemic route of drug delivery yield suboptimal drug levels with the target ocular tissue. Thus frequent administration is practiced, which is associated with exposure to unnecessarily high drug concentrations that in turn causes systemic local drug-induced toxicity and drug wastage.

To address the issues associated with conventional administration, direct injection of drug formulations to the target tissue using conventional hypodermic needles is sought to be highly effective and, therefore, widely employed in clinical treatment of a number of ocular conditions such as CNZ, fungal keratitis, AMD, DM and DME. Direct injection at the disease site offers potential advantages such as overcoming ocular barrier function, the requirement of less amount of drug, instant delivery at the site of action and timely therapeutic benefits. Nevertheless, the long hypodermic needles are associated with a number of issues such as increase in IOP, retinal detachment, discomfort and pain, haemorrhage, likelihood of infections (e.g. endophthalmitis), and need for experienced personnel to administer the injections. Besides, precise anterior segment injections in cornea and sclera, often less than 1000 μm in thickness, using long hypodermic needles is extremely challenging. Use of hypodermic needles is associated with higher degree of tissue trauma.

To overcome both technical and clinical challenges associated with hypodermic needle-based injections, a less invasive mode of treatment is highly desirable. In this regard, researchers found that the use of MN for ocular applications to be an excellent alternative. It is importantly due to the fact that the MNs have successfully demonstrated not just enhanced transdermal drug delivery for the past 10-15 years [65], but also demonstrated its ability to cause significantly less pain [81] and has, therefore, ability to enhance patient compliance [82]. Therefore, translating the benefits of minimally-invasive MNs for ocular applications has been pursued since last 10 years. Importantly, due to the micron-sized of MN, damage to the tissue and

discomfort/pain can be significantly minimized and allow précised localisation of formulation, compared to conventional needles.

In treating anterior segment diseases, the major advantage that MNs hold when compared to traditional topical eye drops is to avoid the major barriers to topical ophthalmic drug delivery, e.g. tear fluid and corneal epithelium. Furthermore, micron-sized tips allow highly localized delivery of drugs compared to traditional topical eye drops. Since the drug is directly delivered to the targeted site, dosage requirement can be minimized with enhanced bioavailability. For example, a 45-fold increase in pilocarpine bioavailability was noted when compared to topical application to the eye [49] and a dose of just 4.4 µg of bevacizumab *via* coated MNs was required, when compared to 52,500 µg delivered *via* eye drops [60]. This indicates significant benefits to the treatment of ocular conditions using MNs, with the added advantage of being minimal tissue damage. Importantly, decreasing the dosage amount will be significant cost savings for some expensive medicines, such as anti-VEGF drug ranibizumab, which has been indicated for topical application for patients suffering from CNZ. Likewise delivery of other anti-VEGFs and gene therapy could save treatment costs, and less dosage can reduce side effects. Furthermore, injecting significantly small volumes (<10 µL) within thin tissues, such as in cornea, using MNs is highly feasible than conventional needles. Tissue damage and recovery will be faster following MN application when compared to hypodermic needles, which in turn will reduce chances of unwanted infections. Therefore, MNs can provide distinct advantages over topical, subconjunctival and other modes of anterior segment drug delivery.

For posterior segment delivery, MNs could offer potential advantages too. Importantly, patients suffering from AMD, DME, retinal vascular occlusions and other retinal disorders require frequent IVT of anti-VEGF agents or corticosteroids for long-term. Despite encouraging outcomes in improving the vision, the frequent use of highly-invasive IVTs has been challenging due to a number of devastating side effects and poor patient compliance. Although the risk of losing vision is more frightening, the anxiety and fear that patients commonly have during hypodermic needled-based IVTs is high. Using significantly shorter MNs can overcome this issue, as demonstrated by a number of studies above.

Periocular injections using conventional needles could overcome side effects due to IVTs. However, due to limited space and very thin tissues (i.e. sclera, SCS), precise injections of drug formulations within the tissues is highly impossible and technically challenging. Therefore, surgical intervention has been employed to administer drug formulations/devices within the tissue [77-79]. But surgical intervention could only add to additional side effects and costs. A number of studies have demonstrated that the posterior segment drug delivery is achievable by delivering small amounts of drug formulations localised within the ocular tissues such as sclera and SCS using MNs. And, due to the shorter length of the MNs, no damage to sensitive tissues such as retina was noticed.

To date, very little work has been done on MN-based ocular drug delivery compared to transdermal drug delivery; therefore further research is essential to realize the benefits of MNs fully. For example, no data has been reported concerning desired MN injection forces to the eye that are regarded as safe, since eye cannot tolerate high forces due to rise in IOP. Matthew et al 2014 [83] as showed that the force needed to insert the hypodermic needle into various areas of the eye wall varies significantly. The required force to insert a needle through the anterior sclera, adjacent to the limbus, and posterior sclera, adjacent to the optic nerve, was the greatest – measuring around 1.0 N. However, the force required to penetrate the central cornea was significantly lower than all other areas i.e. around 0.5 N except the midline sclera, which requires 0.7 N. Therefore, it is important to understand the desired forces of injection, where the MN design should allow easy insertion and produce minimal discomfort. While various designs of MN have been researched for transdermal applications, further studies are necessary to thoroughly evaluate the design constraints that could possibly hinder MNs performance and efficacy. For example, in terms of the stainless steel solid coated MN, the results showed that MN improved fluorescein ocular delivery remarkably and the drug in the coating layer was dissolved rapidly within 20 seconds. However, only 69% of the applied dose was delivered. The rest of the fluorescein either remained adherent to the MN, which was likely due to the incomplete MN insertion into the tissue; or may have deposited on the sclera surface [49]. Thus highlighting the issues of dosing accuracy and reproducibility. Therefore, special attention should be paid for the insertion time, insertion depth, MN design and

method of application, as these factors are interrelated for effective MN penetration and thereby performance.

Contrary to the solid silicon MNs, hollow glass MNs are intrinsically brittle and can be broken off accidently which can be a cause of concern. Moreover, it has been demonstrated that the hollow MN cannot deliver drug solution without retraction of MN from the sclera at a predetermined rate (e.g. 60 μm increments every 3 min) and to a certain critical distance (around 300 μm). Infusion of drug formulation through the hollow MN also requires certain pressure, which is dependent upon viscosity and geometric properties of the MN and viscoelastic properties of the ocular tissue. Uncontrolled retraction from the sclera could lead complete removal of the MN and leakage of the drug onto the sclera surface affecting the amount of drug delivered into the sclera. Thus, special insertion devices and infusion system are required so as to enable MN-based injections in a controlled manner. On the other hand, use of tissue solubilising enzymes (hyaluronidase) can aid the creation of additional space – to accommodate the drug formulation at the target location, but both short-term and long-term effects of tissue integrity must be taken into considered.

Unlike steel or glass MNs, biocompatible and biodegradable polymeric materials can be used in fabrication of MNs. Polymeric MNs will have the advantage of either being completely soluble within the ocular tissue or remain as a depot for long-term drug delivery. And, due to the same reason, the disposable of polymeric MNs will be less of an issue unlike metal/glass MNs, that will have to perhaps follow similar guidelines to that of hypodermic needles.

Other factors to consider are MNs sterility and mechanical properties. For example, it could be easy and cost-effective to have MNs, that are made form steal or glass, to sterilise in similar fashion to that of hypodermic needles. However, polymeric MNs needs special considerations due to their stability issues to heat and other forms of sterilisation; therefore, may need sterile manufacturing. MNs mechanical strength is also a key for its effective application – e.g. metal or glass MNs can withstand higher forces of application than soluble or polymeric-based MNs. Therefore, it is important to consider factors such as type and design of MNs, type of ocular tissues and forces required, so as to enable us to develop MNs of desired qualities. Application of MNs to the eye is another challenge to be addressed, although it is not as straight forward

as it would be for transdermal application. We have discussed a range of MN applicator designs previously [84]; likewise appropriate MN applicator for the eye should de designed to allow precise injections within a given ocular tissue.

Although at its early stage, MNs have so far demonstrated a minimally-invasive means of localised drug delivery to the eye. However, further research is needed to address some of the key challenges. For example, in a recent Phase 1 study it was found that a hollow MN injection into SCS was more painful than IVT, presumably because of distension caused by the volume of drug injected [85]. Therefore, optimization of MN designs, injection volumes, method of injection/retraction, forces of injection, pressure of infusion and tissue damage needs to be thoroughly investigated. Moreover, MNs can be potentially integrated with sustained drug delivery formulations such as nano-/micro-particles, *in situ* forming injectable implants and drug suspension/solution, so as to allow targeted delivery of the formulation within the desired ocular tissue to enable long-term drug delivery. Finally, MNs has the potential to revolutionise ocular drug delivery, as it achieved with transdermal drug delivery. However, this will be highly depended upon the translation of its benefits from lab to the clinic, since to date only one clinical trial is ongoing in this area.

Funding

This paper was not funded

Declaration of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. http://www.who.int/mediacentre/factsheets/fs282/en/ [Last accessed 18 March 2016]
2. Yellepeddi VK, Sheshala R, McMillan H, et al. Punctal plug: a medical device to treat dry eye syndrome and for sustained drug delivery to the eye. Drug Discov Today 2015; 20(7): 884–9
3. Idrees F, Vaideanu D, Fraser SG, et al. A review of anterior segment dysgeneses. Survey Ophthalmol 2006; 51(3): 213–31
4. Geroski DH, Edelhauser HF. Transscleral drug delivery for posterior segment disease. Adv Drug Deliv Rev 2001; 52: 37–48
5. Lee SS, Hughes P, Ross AD, et al. Biodegradable Implants for Sustained Drug Release in the Eye. Pharm Res 2010; 27: 2043–53
6. Ranta VP, Urtti A. Transscleral drug delivery to the posterior eye: Prospects of pharmacokinetic modeling. Adv Drug Deliv Rev 2006; 58: 1164–81
7. Ghate D, Edelhauser HF. Ocular drug delivery. Expert Opin Drug Deliv 2006; 3: 275–87
8. http://www.biographixmedia.com/human/eye-anatomy.html [Last accessed 18 March 2016]
9. Lee VHL, Robinson JR. Topical Ocular Drug Delivery: Recent Developments and Future Challenges. J Ocular Pharmacol 1986; 2: 67–108
10. Lang JC. Ocular drug delivery: conventional ocular formulations. Adv Drug Deliv Reviews 1995; 16: 39–43
11. Geroski DH, Edelhauser HF. Drug Delivery for Posterior Segment Eye Disease. Invest Ophthalmol Vis Sci 2000; 41: 961–64
12. Urtti A, Salminen L. Minimizing systemic absorption of topically administered ophthalmic drugs. Survey Ophthalmol 1993; 37(6): 435–56
13. Urtti A. Challenges and obstacles of ocular pharmacokinetics and drug delivery. Adv Drug Deliv Rev 2006, 58, 1131–5
14. Mikkelson TJ, Chrai SS, Robinson JR. Competitive inhibition of drug-protein interaction in eye fluids and tissues. J Pharm Sci1973; 62: 1942-5
15. J. Fischbarg, The Biology of the Eye, Elsevier, Burlington, 2006

16. Wilson SA, Last A. Management of corneal abrasions, Am. Fam. Physician 2004; 70: 123-8

17. Gaudana R, Ananthula HK, Parenky A, et al. Ocular Drug Delivery, The AAPS Journal 2010; 12:348-60

18. Willoughby CE, Ponzin D, Ferrari S, et al. Anatomy and physiology of the human eye: effects of mucopolysaccharidoses disease on structure and function ? A review, Clin. Experiment. Ophthalmol 2010; 38: 2-11

19. Schoenwald RD, Ward RL. Relationship between steroid permeability across excised rabbit cornea and octanol-water partition coefficients. J Pharmaceutical Sciences 1978; 67: 786–88

20. Sasaki H, Ichikawa M, Yamamura K et al. Ocular membrane permeability of hydrophilic drugs for ocular peptide delivery. The J Pharma Pharmacol 1997; 49: 135–9

21. Bachman WG, Wilson G. Essential ions for maintenance of the corneal epithelial surface. Invest Ophthalmol Vis Sci 1985; 26:1484–8

22. Akhtar S, Tullo A, Caterson B, et al. Clinical and morphological features including expression of betaig-h3 and keratan sulphate proteoglycans in Maroteaux-Lamy syndrome type B and in normal cornea, Br. J. Ophthalmol 2002; 86:147-51

23. Malhotra M, Majumdar DK. Permeation through cornea, Indian. J. Exp. Biol 2001; 39:11-24

24. Rabinovich-Guilatt L, Couvreur P, Lambert G, et al. Cationic Vectors in Ocular Drug Delivery. J Drug Targeting 2004; 12: 623–33

25. Graymore CN. Biochemistry of the Eye. Academic Press, London, New York, p.19. 1970

26. Kompella UB, Kadam RS, Lee VHL. Recent advances in ophthalmic drug delivery. Therapeutic delivery 2010; 1: 435–56

27. Olsen TW, Aaberg SY, Geroski DH, et al. Human sclera: thickness and surface area, Am. J. Ophthalmol 1998; 125: 237-41

28. Dunlevy JR. Summers RJ. Interaction of lumican with aggrecan in the aging human sclera. Invest Ophthalmol Vis Sci 2004; 45(11): 3849–56

29. Watson PG, Young RD. Scleral structure, organisation and disease. A review, Expt. Eye Res 2004; 78: 609-23

30. Ambati J, Canakis CS, Miller JW, et al. Diffusion of high molecular weight compounds through sclera. Invest Ophthalmol Vis Sci 2000, 41, 1181–5

31. Edwards A, Prausnitz MR. Fiber matrix model of sclera and corneal stroma for drug delivery to the eye. AIChE J 1998; 44: 214–225

32. Kuno N, Fujii S. Recent Advances in Ocular Drug Delivery Systems. Polymers 2011; 3: 193–221

33. Ikuno Y, Kawaguchi K, Nouchi T, et al. Choroidal Thickness in Healthy Japanese Subjects RID F-2586-2011. Invest Ophthalmol Vis Sci 2010; 51: 2173–76

34. Margolis R, Spaide RF. A Pilot Study of Enhanced Depth Imaging Optical Coherence Tomography of the Choroid in Normal Eyes. Am J Ophthalmol 2009; 147: 811–5

35. Chong NHV, Keonin J, Luthert PJ, et al. Decreased thickness and integrity of the macular elastic layer of Bruch's membrane correspond to the distribution of lesions associated with age-related macular degeneration. Am J Pathol 2005; 166: 241–51

36. Hornof M, Toropainen E, Urtti A. Cell culture models of the ocular barriers. Euro J Pharma Biopharma 2005; 60: 207–25

37. Heiduschka P, Fietz H, Hofmeister S, et al. Penetration of bevacizumab through the retina after intravitreal injection in the monkey. Invest Ophthalmol Vis Sci 2005; 48: 2814–23

38. Galloway NR, Amoaku WMK, et al. Common Eye Diseases and their Management, Springer, 2006.

39. Yoeruek E, Ziemssen F, Henke-Fahle S, et al. Safety, penetration and efficacy of topically applied bevacizumab: evaluation of eyedrops in corneal neovascularization after chemical burn. Acta Ophthalmologica 2007; 86: 322-8

40. Kim WJ, Jeong HO, Chung SK. The Effect of Bevacizumab on Corneal Neovascularization in Rabbits. Korean J Ophthalmol 2010; 24: 230–7

41. Chang JH, Garg NK, Lunde E, et al. Corneal Neovascularization: An Anti-VEGF Therapy Review. Survey Ophthalmol 2012; 57: 415–29

42. Dastjerdi MH, Al-Arfaj KM, Nallasamy N, et al. Topical bevacizumab in the treatment of corneal neovascularization: results of a prospective, open-label, noncomparative study. Arch Ophthalmol 2009; 127: 381–9

43. Vieira ACC, Höfling-Lima AL, Gomes JÁP et al. Intrastromal injection of bevacizumab in patients with corneal neovascularization. Arquivos Brasileiros De Oftalmologia 2012; 75: 277–9

44. Gaudana R, Ananthula HK, Parenky A, et al. Ocular Drug Delivery. The AAPS J 2010; 12: 348–60

45. Hashemian MN, Zare MA, Rahimi F, Mohammadpour M. Deep intrastromal bevacizumab injection for management of corneal stromal vascularization after deep anterior lamellar keratoplasty, a novel technique, Cornea 2011; 30: 215-8

46. Sharma N, Agarwal P, Sinha R, et al. Evaluation of intrastromal voriconazole injection in recalcitrant deep fungal keratitis: case series, Br. J. Ophthalmol 2001; 95: 1735-7

47. Kalaiselvi G, Narayana S, Krishnan T, et al. Intrastromal voriconazole for deep recalcitrant fungal keratitis: a case series. Br J Ophthalmol 2015; 99: 195–8.

48. Maurice D. Review: practical issues in intravitreal drug delivery, J. Ocul. Pharmacol Ther 2001; 17: 393-401.

49. Jiang J, Gill HS, Ghate D, et al. Coated microneedles for drug delivery to the eye, Invest. Ophthalmol Vis Sci 2007; 48: 4038-43.

•• First proof of concept of ocular application of microneedles

50. You X, Li J, Li S, et al. Effects of Lamellar Keratectomy and Intrastromal Injection of 0.2% Fluconazole on Fungal Keratitis, J. Ophthalmol. 2015; 656027

51. Ohm J. Über die Behandlung der Netzhautablösung durch operative Entleerung der subretinalen Flüssigkeit und Einspritzung von Luft in den Glaskörper. Albrecht von Græfe's Archiv für Ophthalmologie 1911; 79: 442–50. **• Early evidence of intravitreal injections**

52. Ramulu PY, Do DV, Corcoran KJ, et al. Use of retinal procedures in medicare beneficiaries from 1997 to 2007. Arc Ophthalmol 2010; 128: 1335–40

53. Amoaku W. The Royal College of Opthamologists Audit: Summary of Survey on Provision of AMD Services, 2009

54. Englander M, Chen TC, Paschalis EI, et al. Intravitreal injections at the Massachusetts Eye and Ear Infirmary: analysis of treatment indications and postinjection endophthalmitis rates. Br J Ophthalmol 2013; 97: 460–5

55. The Royal College of Opthamologists, 2009. Guidelines for Intravitreal Injections procedure. Available at: https://www.rcophth.ac.uk/wp-content/uploads/2015/01/2009-SCI-012_Guidelines_for_Intravitreal_Injections_Procedure_1.pdf [Last accessed 18 March, 2016]

• **Guidelines for intravitreal injections in the UK**

56. Del Amo EM, Urtti A. Current and future ophthalmic drug delivery systems. A shift to the posterior segment. Drug Discov Today 2008; 13:135–43

57. Kurz D, Ciulla TA, Novel approaches for retinal drug delivery. Ophthalmol Clinics North Am 2002; 15: 405–10

58. Falavarjani KG, Nguyen QD, Adverse events and complications associated with intravitreal injection of anti-VEGF agents: a review of literature. Eye 2013; 27: 787–94

59. Peyman GA, Lad EM, Moshfeghi DM. Intravitreal injection of therapeutic agents. Retina 2009; 29: 875–912

60. Kim YC, Grossniklaus HE, Edelhauser HF, et al. Intrastromal delivery of bevacizumab using microneedles to treat corneal neovascularization, Invest. Ophthalmol. Vis. Sci 2014; 55: 7376-86

•• **MN-based intrastromal injections of anti-VEGFs**

61. Fattal E, Bochot A. Ocular delivery of nucleic acids: antisense oligonucleotides, aptamers and siRNA, Adv Drug Deliv Rev 2006; 58:1203-23

62. Donnelly RF, Thakur RRS, Garland MJ,□et al. Hydrogel-forming microneedle□arrays for enhanced transdermal drug□delivery. Adv Funct Mater 2012;22: 4879-90.

63. Henry S, McAllister DV, Allen MG, et al. Microfabricated microneedles: a novel approach to transdermal drug delivery. J Pharm Sci 1998;87:922-5

64. Roxhed N, Samel B, Nordquist L, et al. Painless drug delivery through microneedle-based transdermal patches featuring active infusion. IEEE Transactions Bio-med Eng 2008; 55: 1063–71.

65. Donnelly RF, Thakur RRS, Morrow DIJ et al. Microneedle-mediated Transdermal and Intradermal Drug Delivery. Wiley- Blackwell, Oxford, UK, 2012. **•• First book on microneedles – recommended for further information on MNs e.g. fabrication methods**

66. Quinn HL, Kearney MC, Courtenay AJ et al. The role of microneedles for drug and vaccine delivery. Expert Opin Drug Deliv 2014; 11:1769-80

67. Thakur RRS, Fallows SJ, McMillan HL, et al. Microneedle-mediated intrascleral delivery of in situ forming thermoresponsive implants for sustained ocular drug delivery. J Pharmacy Pharmacol 2014; 66: 584–95

•• First MN-based paper that demonstrated intrascleral injection of in situ implant forming gels

68. Jiang J, Moore JS, Edelhauser HF, et al. Intrascleral Drug Delivery to the Eye Using Hollow Microneedles. Pharma Res 2008; 26: 395–403

69. Patel SR, Lin ASP, Edelhauser HF, et al. Suprachoroidal Drug Delivery to the Back of the Eye Using Hollow Microneedles. Pharma Res 2011; 28: 166–76.

**** First proof of concept of paper for suprachoroidal injection using microneedles**

70. Patel SR, Berezovsky DE, McCarey BE, et al. Targeted administration into the suprachoroidal space using a microneedle for drug delivery to the posterior segment of the eye. Invest Opthalmol Vis Sci 2012; 53: 4433–41

71. Gilger BC, Abarca EM, Salmon JH, et al. Treatment of acute posterior uveitis in a porcine model by injection of triamcinolone acetonide into the suprachoroidal space using microneedles. Invest Opthalmol Vis Sci 2013; 54: 2483–92

72. Chiang B, Yoo CK, Edelhauser HR, et al. Circumferential flow of particles in the suprachoroidal space is impeded by the posterior ciliary arteries. Expt Eye Res 2016; 1–35

73. Song HB, Lee KJ, Ho Seo, et al. Impact insertion of transfer-molded microneedle for localized and minimally invasive ocular drug delivery. J Control Rel 2015; 209: 272–9

74. Palakurthi NK, Correa ZM, Augsburger JJ, et al. Toxicity of a biodegradable microneedle implant loaded with methotrexate as a sustained release device in normal rabbit eye: a pilot study. J Ocular Pharmacol Thera 2011; 27: 151–6

75. Jiang JW, Joshi M, Christoforidis J. Drug Delivery Implants in the Treatment of Vitreous Inflammation. Mediators of Inflammation 2013;2013: 1–8

76. Yasukawa T, Kimura H, Tabata Y, et al. Biodegradable scleral plugs for vitreoretinal drug delivery. Adv Drug Deliv Rev 2001; 52: 25-36

77. Okabe J. Biodegradable Intrascleral Implant for Sustained Intraocular Delivery of Betamethasone Phosphate. Invest Ophthalmol Vis Sci 2003, 44: 740–4

78. Shin JPJ, Park YCY, Oh JHJ, et al. Biodegradable intrascleral implant of triamcinolone acetonide in experimental uveitis. J Ocular Pharmacol Ther 2009; 25: 201–8

79. Kim Y, Lim J, Kim H, et al. A novel design of one-side coated biodegradable intrascleral implant for the sustained release of triamcinolone acetonide. European J Pharma Biopharma 2008; 70: 179–86

80. https://clinicaltrials.gov/ct2/results?term=ocular+microneedle&Search=Search [Last accessed 18 March 2016]

81. Gill HS, Denson DD, Burris BA, et al. Effect of microneedle design on pain in human subjects. Clin J Pain 2008; 24: 585–94

82. Mooney K, McElnay JC, Donnelly RF. Paediatricians' opinions of microneedle-mediated monitoring: a key stage in the translation of microneedle technology from laboratory into clinical practice. Drug Deliv Transl Res 2015;5: 346-59

83. Matthews A, Hutnik C, Hill K, et al. Indentation and needle insertion properties of the human eye. Eye 2014; 28: 880-7

84. Thakur RRS, Dunne NJ, Cunningham E, et al. Review of patents on microneedle applicators. Recent Pat Drug Deliv Formul. 2011;5:11-23

85. Goldstein DA. Achieving Drug Delivery Via the Suprachoroidal Space. Retina Today. July/Aug 2014. 82-7

Table 1. Summary of routes of ocular drug delivery. Adapted from [44].

Route	Benefits	Challenges
Topical	Patient compliance, self-administration, non-invasive	Tear dilution and turnover, corneal barrier, efflux pumps, <5% bioavailability
Oral/systemic	Patient compliance, non-invasive	Blood-aqueous barrier, blood-retinal barrier, high dosing causes toxicity, <2% bioavailability
Intravitreal	Direct delivery to vitreous and retina, sustained drug levels, evades blood-retina barrier	Retinal detachment, haemorrhage, cataract, endophtalmitis, patient incompliance
Intracameral	Higher drug levels in anterior chamber, eliminates use of drops, reduces corneal and systemic side effects seen with topical steroid therapy	Toxic anterior segment syndrome, toxic endothelial cell destruction syndrome
Subconjunctival	Anterior and posterior delivery, potential for depot formulations	Conjunctival and corneal circulation
Subtenon	High vitreal drug levels, relatively non-invasive, fewer complications than intravitreal	Retinal pigmented epithelium, chemosis, subconjunctival haemorrhage
Retrobulbar	High local doses of anaesthetics, more effective than peribulbar, minimal effect on intraocular pressure	Retrobulbar haemorrhage, glober perforation, respiratory arrest
Posterior juxtascleral	Safe for depot delivery, sustained drug levels for up to 6 months to macula, avoids risk of endophtalmitis and intraocular damage	Surgery, retinal pigmented epithelium acts as barrier.

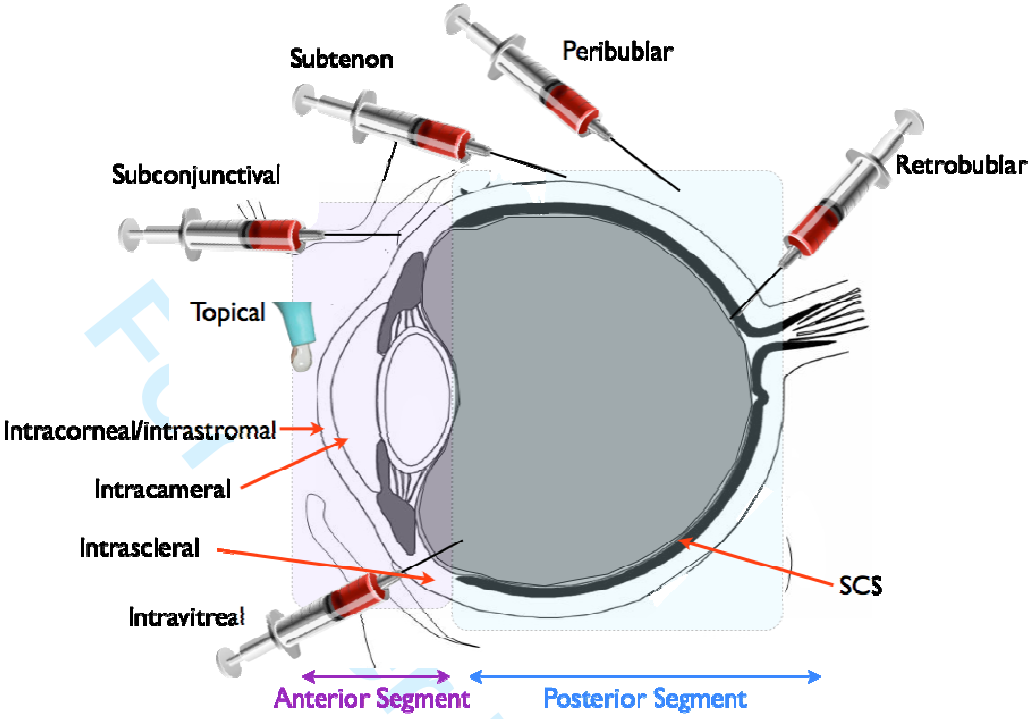

Figure 1. Schematic representation showing various routes of ocular drug delivery.

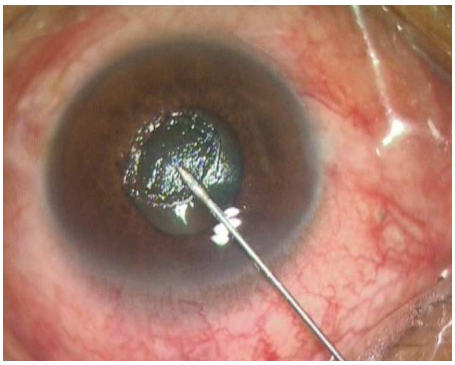

Figure 2. Digital photograph showing intrastromal injection of fluconazole using hypodermic needle to treat fungal keratitis. Adapted from [50].

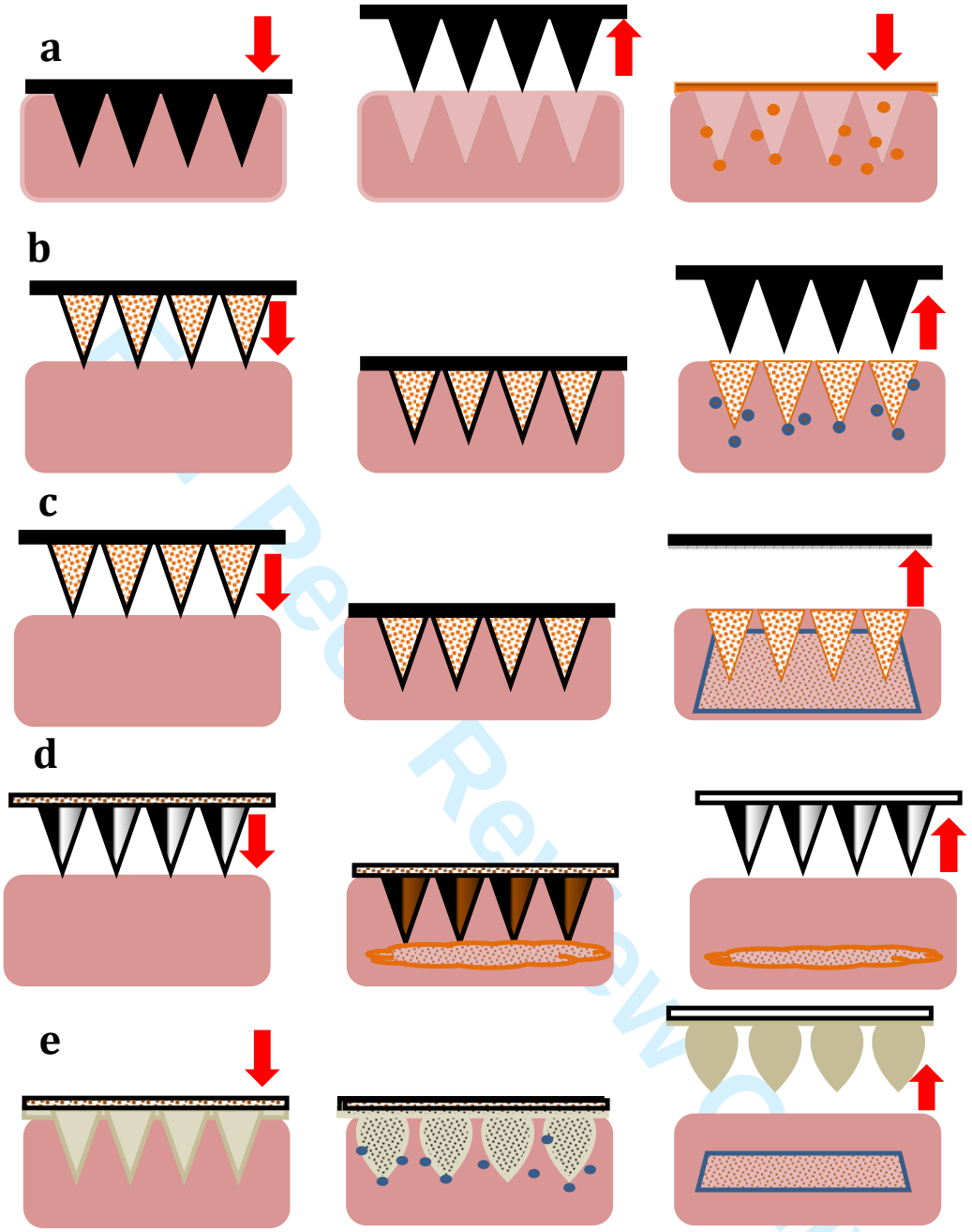

Figure 3. Schematic representation of different modes of MN application. (a) *Poke and patch* – application and removal of solid MNs and followed by application of drug-loaded reservoir. (b) *Coat and poke* – application of coated MNs for deposition of drug-containing layer in the skin. (c) Application of dissolving MNs (made of polymer or sugar) for delivery of incorporated drug into the skin. (d) Injection of drug formulation using hollow MNs. (e) Application of swelling MNs for drug delivery through the hydrogel matrix from a drug-loaded reservoir [66].

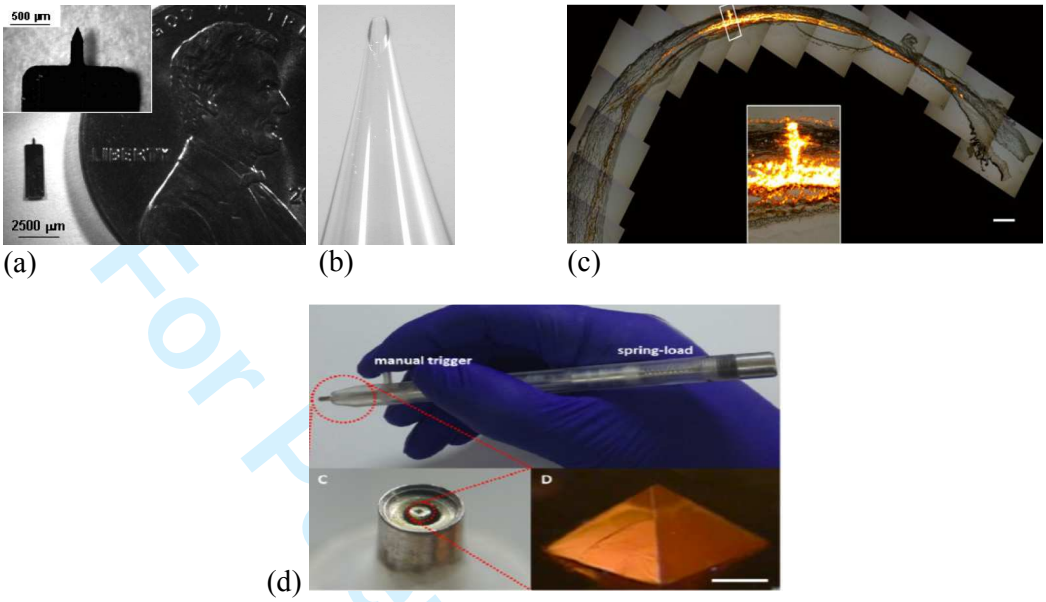

Figure 4. (a) Microscopic image of a single solid stainless-steel MN used for intrascleral and intracorneal administration shown next to a US penny. The inset shows magnified view of the MN, which is 500 µm in length and 45° in tip angle [49]. (b) Representative glass-based HMN with a bevel tip angle of 25° [68] (c) Image showing 1000 nm particles distribution into the SCS of human eye, *ex vivo*. The inset represents a magnified view of the HMN insertion site. Scale bar is 500 µm [69]. (d) Shows a photograph of; (i) spring-loaded MN pen; (ii) MN guiding structure at the end of MN pen and (iii) transfer molded MN structure on the tip end of MN pen. Scale bar is 100 µm [73].

a. Reproduced with permission from [49].
b. Reproduced with permission from [68].
c. Reproduced with permission from [69].
d. Reproduced with permission from [73].

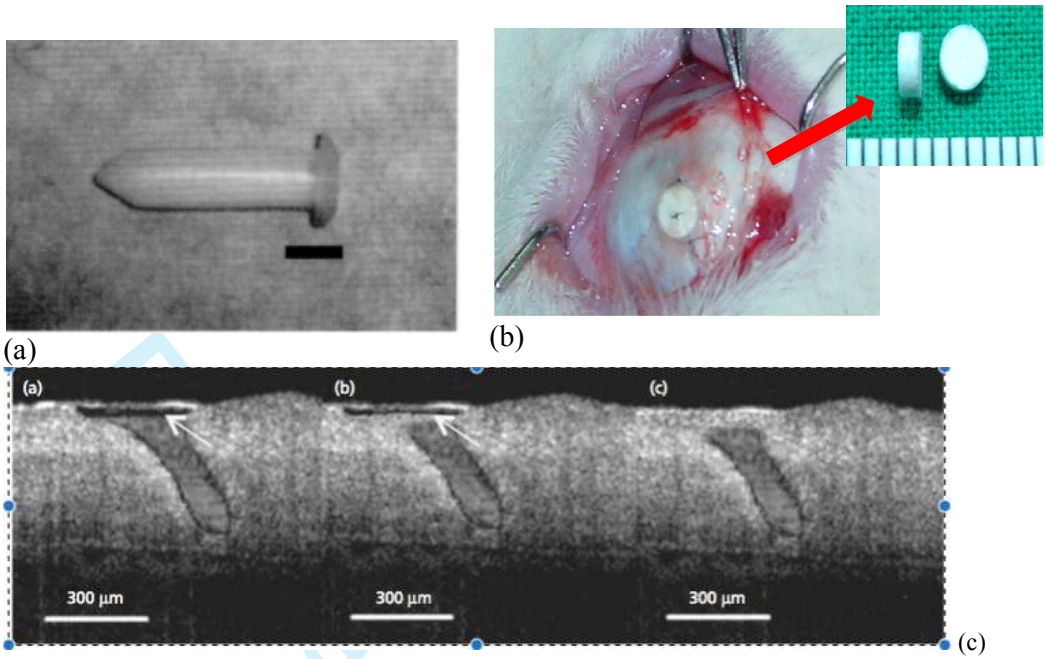

Figure 5. (a) Shows digital image of a biodegradable scleral plug containing that is 5 mm in length and 1 mm in diameter [76]. (b) Image of intrascleral implant, in rabbit eye, at the site of surgical administration one week after the implantation. The inset shows the biodegradable one-side coated triamcinolone acetonide intrascleral implant with 1 mm in thickness and 3 mm in diameter [79]. (c) Optical coherence tomography images showing 30 G HMN injection of 50 µl fluorescein sodium-loaded poloxamer gel injected into equatorial sclera to a depth of 400 µm at *(a)* 0, and *(b)* 1 and *(c)* 2 h after injections, where the arrow indicates empty space in sclera created following HMN application and its subsequent closure over time [67].

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b. Reproduced with permission from [79].
c. Reproduced with permission from [67].