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Microneedles for advanced ocular drug delivery

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Abstract

In the field of ocular drug delivery, topical delivery remains the most common treatment option for managing anterior segment diseases, while intraocular injections are the current gold standard treatment option for treating posterior segment diseases. Nonetheless, topical eye drops are associated with low bioavailability (<5%), and the intravitreal administration procedure is highly invasive, yielding poor patient acceptability. In both cases, frequent administration is currently required. As a result, there is a clear unmet need for sustained drug delivery to the eye, particularly in a manner that can be localised. Microneedles, which are patches containing an array of micron-scale needles (< 1 mm), have the potential to meet this need. These platforms can enable localised drug delivery to the eye while enhancing penetration of drug molecules through key ocular barriers, thereby improving overall therapeutic outcomes. Moreover, the minimally invasive manner in which microneedles are applied could provide significant advantages over traditional intravitreal injections regarding patient acceptability. Considering the benefits of this novel ocular delivery system, this review provides an in-depth overview of the microneedle systems for ocular drug delivery, including the types of microneedles used and therapeutics delivered. Notably, we outline and discuss the current challenges associated with the clinical translation of these platforms and offer opinions on factors which should be considered to improve such transition from lab to clinic.

Keywords: Microneedle, ocular, computational modelling, hollow microneedle, solid microneedle

Graphical Abstract:

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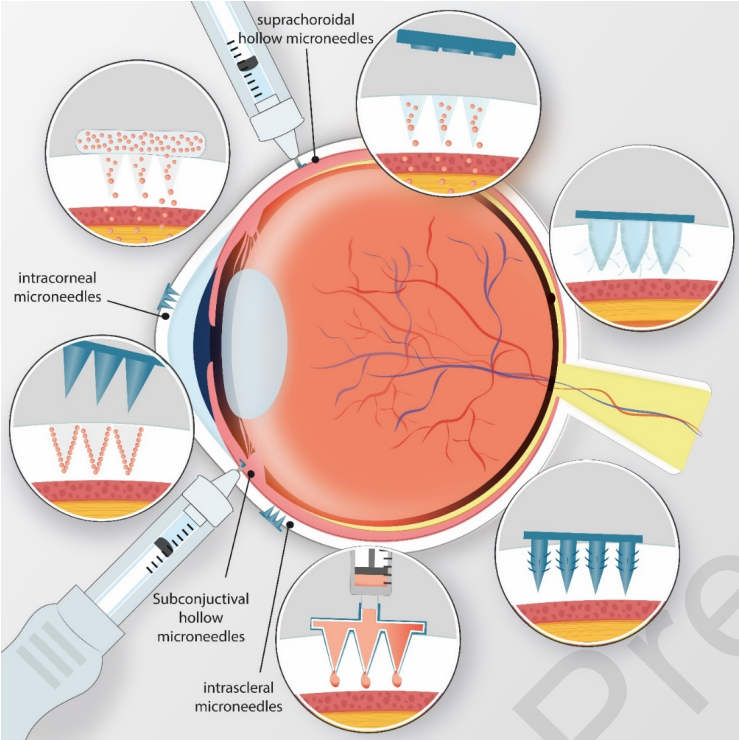
1. Introduction

1.1. Challenges in ocular drug delivery

As of the body's smallest yet perhaps most complex organ, [1]the eye is crucial to our quality of life. The role it plays in capturing sensory information in the form of vision, before passing it to the brain for processing, enables us to interact with the world on a daily basis.

According to reports published by the World Health Organization, more than 2.2 billion people are affected with some degree of visual impairment, which can be described as a deviation from 'perfect' vision. These deviations, typically occurring as a result of ocular diseases or conditions, can range from minor vision disturbances to complete blindness. Notably, approximately half of these cases are estimated to be preventable form progressing to blindness [2].

Conditions such as myopia and presbyopia, despite demonstrating high global prevalence, can be often corrected by glasses or contact lenses. More severe conditions, such as age-related macular degeneration (AMD) and diabetic retinopathy; however, require a more robust and structured treatment approach over a long-term period (months to years). Given that approximately 196 and 146 million people worldwide are estimated to be suffering from AMD and diabetic retinopathy respectively [2], this undoubtedly has the potential to place a burden on healthcare systems.

[2]Chronic ocular diseases affecting the anterior segment (e.g., glaucoma and amebic keratitis) and posterior segment (e.g., AMD and diabetic retinopathy) typically require long-term treatment, in the region of months to years [3,4]. While topical drug delivery remains the ideal treatment option for the management of anterior segment ocular diseases that require only short-term treatment , this approach tends to be less efficacious for the treatment of long-term anterior segment diseases or back of the eye diseases, such as AMD. This can be owed to the fact that eye drops incur several drawbacks, including but not limited to low bioavailability (< 5%), nasolacrimal drainage, systemic absorption, and lymphatic drainage [5], and thus it is challenging to achieve and sustain therapeutic levels at the retina using this approach.

[3,4][6]On the other hand, treatment for disease conditions affecting the posterior segment of the eye often requires intraocular injections, with intravitreal injections of anti-VEGF agents (e.g., bevacizumab, ranibizumab, etc.) being the current gold standard. However, the unique vitreoretinal barriers that exist can prevent the entry of large molecules into the target retinal pigment epithelium cells, still making it challenging to deliver effective treatment [7] Moreover, intravitreal injections incur several limitations that have been well established in literature, including inducing endophthalmitis, pseudo endophthalmitis and vitreous detachment. The repeated administration of these injections, typically on a monthly basis, leads to potential summation of the outlined risked for patients requiring these treatments [8] Figure 1 shows a schematic representation of the numerous ocular barriers that play a role in impeding efficacious drug delivery to the eye.

Even when successfully delivered *via* intravitreal injection, more than approximately 45% of AMD patients do not respond to anti-vascular endothelial growth factor (VEGF) drugs [9].

Furthermore, age-related transformations in the vitreous humor can lead to alterations in the flow dynamics of formulations within the eye, which poses additional challenges for efficacious posterior segment delivery [10]. Liquification of vitreous humor with age is also related to complications such as vitreous detachment, macular holes, and hemorrhage. This, combined with poor permeation of anti-VEGF drugs due to their high molecular weight, short half-life and binding to extracellular matrix and blood–retinal barrier, further limits the success of treating posterior segment diseases by decreasing the predictability and reproducibility of intravitreal pharmacokinetics.

As outlined, current treatment strategies for anterior and posterior segment diseases have significant drawbacks, highlighting the need for innovative approaches. In particular, localised delivery has the potential to provide therapeutic benefits by preventing systemic side effects, while also improving clinical outcomes such as effective treatment of diseases, improved patient compliance and reduce economic burdens [8].
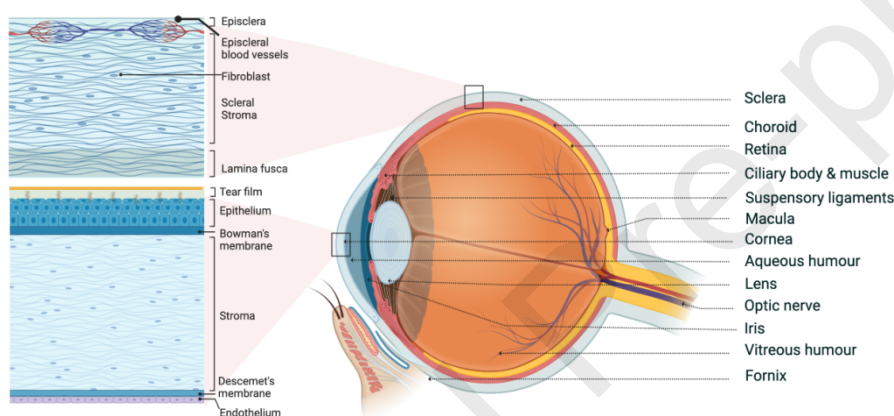

Figure 1. Schematic representation of tissue barriers to ocular drug formulations.

To overcome the limitations of current approaches of ocular drug delivery, researchers are exploring various strategies to improve the bioavailability of ocular drugs. This includes the use of drug delivery systems such as nanoparticles, liposomes, and hydrogels, as well as prodrugs, permeation enhancers, or device-based (e.g., iontophoresis, Micro-Electro-Mechanical Systems) strategies that can enhance drug penetration and retention in the eye. Meanwhile, targeted drug delivery systems can improve the pharmacokinetics of drugs by delivering cargo directly to affected cells, such as within the retina or iris, rather than relying on diffusion and permeation processes post-administration.

1.2. Localised drug delivery to improve ocular outcomes?

Localised drug delivery systems can be utilized to achieve higher bioavailability at specific regions or tissues of the eye. Microneedles are one such platform that can be designed to deliver drug payloads directly to the target tissue, thereby improving the efficacy of treatment and minimizing potential side effects associated with non-specific drug distribution.

Microneedles are minimally invasive drug delivery devices capable of localized and prolonged drug delivery for the management of chronic diseases. According to the FDA, "microneedling devices create many small puncture holes in the skin" [11]. Over the past decade, various types

of microneedles have been developed for specific clinical conditions, from drug delivery to aesthetics. As will be discussed further within this review, the main types of microneedles include solid, dissolving, coated, hydrogel-forming and hollow microneedles.

It must be noted however that complex yet delicate organs such as eye can be a challenging site for microneedle administration. The variability of biomechanical properties of ocular tissues, sensitivity to pressure changes and limited surface area for drug deposition are just a few of the hurdles which must be overcome to achieve effective drug delivery to the eye using microneedles. Despite some clinical success [6], there is a long way to go regarding microneedles being a common means for delivering drug to the eye.

This review aims to highlight such challenges and offer perspective on what learnings can be made from existing microneedle research to aid the clinical translation of these platforms for ocular applications. We start however by outlining the history of microneedles, their advantages, and how they have been studied thus far for ocular drug delivery.

1.3. The history of microneedles for drug delivery applications

The first documented evidence of microneedles use was reported by Dr. Ernst Kromayer in 1905, with a report suggesting the application of motorized dental burs for the treatment of scarring and hyperpigmentation. Instead, the delivery of drugs using microneedle platforms began to receive more attention in the 1960s. This has been accompanied by achievement of several milestones in the application of various materials, manufacturing methods and types of microneedles [12].

The utilisation of microneedles has been experimental in the context of localised drug delivery and targeting, primarily within the field of transdermal drug delivery. This can be owed to their ability to be tailored to penetrate the stratum corneum to enhance the permeability of molecules across the skin. As previously elucidated, various microneedle types have been employed for ocular purposes, mainly solid, hollow, and dissolving microneedles. Overall, microneedles have the potential to improve drug delivery, localization and targeting, especially for drugs that are difficult to deliver using traditional methods.

As of 2021, 73.4% of papers reporting on microneedles were related to delivery applications [13]; however, the use of microneedles is not solely limited to drug delivery applications. These platforms have also been exploited for diagnostic [14], and cosmetic applications [15]. Among the papers published to date on microneedles, solid microneedles accounted for approximately one-third of these papers, with the most common therapeutics delivered (32%) being small molecules [13]

Prof. Mark Prausnitz's group suggested the first use of microneedles for ocular applications in the early 2000s. The paper suggested use of a coated microneedle for the therapeutics delivery of protein and DNA to the eye. Various groups then used hollow microneedles and soluble microneedles for the administration of ophthalmic drugs. According to current PubMed publication trends, there has been an increase in interest concerning the development of microneedles for ocular administration (Figure 2) [16]
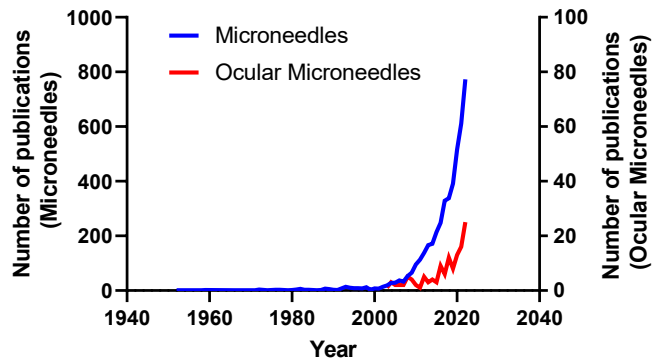
Figure 2. Trends in research and review publication in field of microneedles and ocular microneedles from 1950 to 2022 as per PubMed.

1.4. Advantages of microneedles for ocular drug delivery

In contrast to intraocular injections using conventional hypodermic needles (> 10 mm in length), microneedles (< 1 mm in length) can be applied in a minimally invasive manner to the eye, leading to lower tissue trauma and more localized tissue-specific drug delivery [17]. Given the unique features of microneedle-based drug delivery systems, they offer several advantages when compared to other ocular drug delivery systems. The available routes for microneedle administration to the eye have been shown in Figure 3.

Figure 3. Schematic representation of the eye demonstrating the available route for microneedle administration, taken from Vora et al [18].

One of the primary advantages microneedles offer is the ability to overcome physiological barriers. Figure 4a shows bilayer dissolving microneedle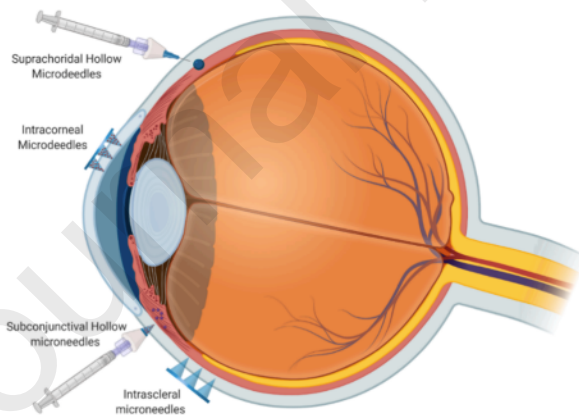s developed by Wu et al., which achieved 76 % insertion of the total needle length into porcine sclera under a force of 3 N. Furthermore, within just 24 hours, the OVA-loaded nanoparticles had distributed to the bottom of the sclera [19]. Crucially, since microneedles mechanically overcome barrier functions of the ocular tissues, the physicochemical properties of the drug that normally govern drug partition across tissues (i.e., pKa, log P, molecular weight, solubility, among others) are less critical to successful penetration. This makes microneedle platforms a viable strategy for the delivery of a wide variety of therapeutic agents, as will be outlined later in the review.

Localised drug delivery is another key benefit that microneedle-based devices offer in comparison to intravitreal injections for the treatment of posterior segment diseases. As shown in Figure 4b. localised delivery of rhodamine dye into the suprachoroidal space was possible using hollow microneedles, due to the highly precise delivery they can enable [20], in comparison a large bolus created by intravitreal injections in the vitreous cavity (Figure 4c).

Additionally, dissolving microneedles have been shown to produce administered drug micro-depots in the target ocular tissue in eye layers. From these depots, drugs can dissolve and release in a sustained manner, with the polymeric components of the dissolving microneedles acting as a sustained release platform. This provides significant advantages compared to conventional formulation strategies, such as topical eye drops, which require frequent administration, both in terms of patient acceptance and in reducing the clinical burden in disease treatment. Furthermore, the potential for successful sustained release of large molecules, such as biologics, has been demonstrated in the sclera using dissolving microneedles [19]

In addition to therapeutic advantages, microneedles also offer clinical advantages. Their smaller size in comparison to hypodermic needles (Figure 4d and e) has the potential to avoid issues regarding needle-phobia associated with procedures such as intravitreal injections.
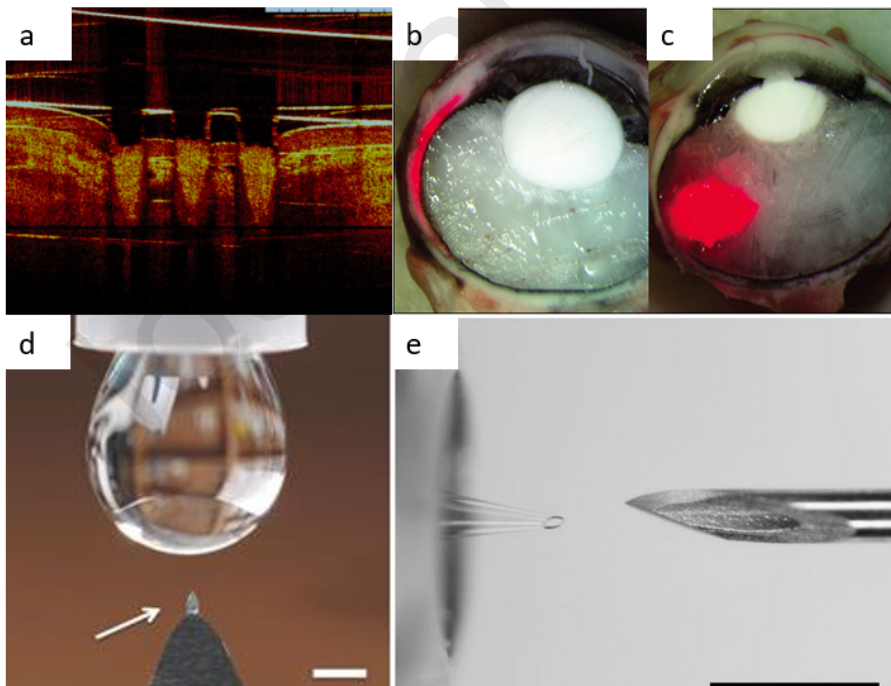
Figure 4. (a) insertion of polymeric bilayer microneedles inserted into porcine sclera captured by OCT [19], (b) distribution of rhodamine dye on suprachoroidal injection and (c) intravitreal injection in *ex vivo* porcine eye [21], (d) comparison of eye drop size from a typical eye drop and a hollow microneedle [22]and (e) comparative image glass hollow microneedle vs hypodermic needle [23].

2. Types of microneedles for ocular drug delivery

Classification of microneedle types is typically established based on their geometry and mechanism of drug delivery. Since research into these platforms into drug delivery began, several microneedle types have been developed and studied: solid, solid-coated, dissolving, hydrogel-forming and bio-inspired microneedles.

Currently, the only types of microneedles have been explored widely for ocular applications: hollow, solid (coated), dissolving microneedles and bio-inspired microneedles (Figure 5). The described microneedles have been utilized to deliver a wide range of formulation types, such as solutions [23], nano/microparticle formulations [23], gene therapies [24] and thermoresponsive polymers [25][17]. Moreover, these platforms have been utilized to target localized delivery of therapeutics to specific tissues within the eye, such as the cornea, sclera, and suprachoroidal space, to provide localized delivery of therapeutics and sustained release formulations, as will be discussed later in the review. This section goes into detail about each microneedle type studied for ocular drug delivery, including their corresponding advantages and limitations, in relation to drug delivery applications.
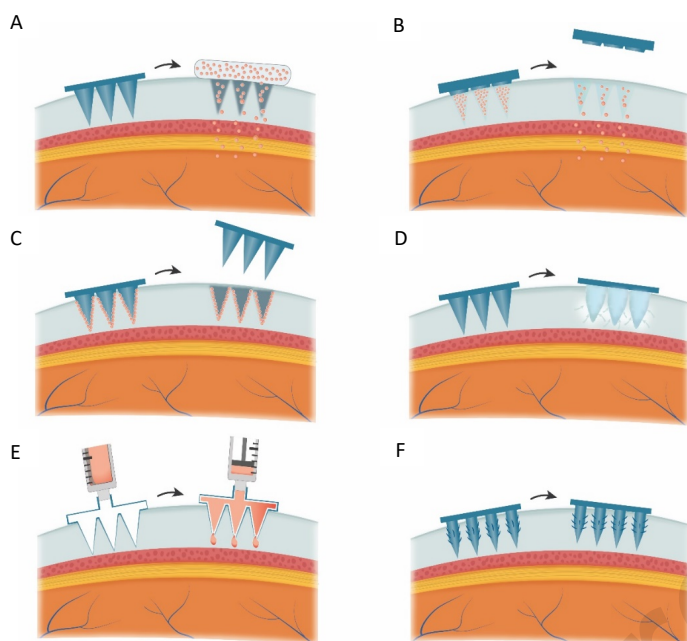
Figure 5. Schematic diagram showing (A) solid microneedles, (B) dissolving microneedles, (C) solid-coated microneedles, (D) hydrogel-forming microneedles, (E) hollow microneedles and (F) bioinspired microneedles.

2.1 Dissolving microneedles

Dissolving microneedles are created by incorporating drug molecules into dissolvable and biocompatible polymers, such as poly(vinyl alcohol) (PVA) and poly(vinyl pyrrolidone) (PVP), hyaluronic acid[26,27]. Following insertion into the target tissue, these polymeric microneedle structures will dissolve, typically within minutes, once in contact with aqueous fluid to release their cargo [27].

The administration of dissolving microneedles to the eye is intended to be simple and patient-friendly, similar to contact lens administration, as it only requires gentle thumb pressure on the ocular surface [28]. Contact lens-based microneedles have also been researched to enhance the penetration of therapeutics in ocular tissues[29] . These types of contact lenses take advantage of both patient familiarity and acceptance of conventional contact lens design in combination with the drug delivery advantages offered by microneedles. This approach has been used to successfully deliver small molecules such as pilocarpine to the anterior segment of the eye [29].

Dissolving microneedles offer several potential advantages for ocular drug delivery applications. Namely, the use of biocompatible polymers that dissolve and are naturally removed from human tissue will not generate biohazardous sharp waste, such as is the case for

solid and hollow microneedles. After administration, the needles themselves will temporarily remain in the tissue, and the baseplate is simply removed from the application site. As dissolving microneedles do not need to be retrieved from the target tissue after insertion, the risk of broken microneedles remaining in the tissue is eliminated, which is a concern with both hollow and solid microneedles. Furthermore, dissolving microneedles offer design flexibility in that various patch sizes and geometries can be fabricated using a relatively straightforward and cost-effective micromold-casting method [30].

Dissolving microneedles have also been shown to sustain the release of therapeutics loaded into the polymer matrix, controlled by optimizing the composition of the polymer matrix. This can be further extended either by choosing slowly dissolving polymers or by entrapping drug in micro/nanoparticles which are then loaded within the needles [31]. In a recent study, Lee et al., designed a dissolving microneedle tip pen with a hybrid microneedle and a drug-loaded detachable tip with a supporting base for testing *ex vivo* microneedle-mediated intrascleral delivery. The attached device is designed to allow the detachment of the drug-loaded tip with the impact insertion mechanism (Figure 6a) [32]

Although dissolving microneedles have a variety of advantages and have been widely used for anterior and posterior ocular drug administration, it is undeniable that this type of microneedle still incurs several limitations. Because of the small size of needles (~ < 1 mm in height) and the localization of the therapeutic exclusively in the needles themselves, the loading capacity is limited [33]. As a result, a large portion of ocular microneedle research has concentrated on the administration of highly potent drugs to ensure target therapeutic levels are achieved. Additionally, patch size is limited by the curvature of the eye, whereby needles on the outermost regions of the patch may not be inserted as effectively due to varying load distributions upon administration.

2.2. Coated microneedles

Coated microneedles work based on the 'coat and poke' strategy, in that therapeutics are precoated onto the tips of the microneedles and then inserted into the target ocular tissue. After insertion, the therapeutic molecules coated on solid microneedles can be immediately deposited at the application site. By bypassing the ocular barrier function, coated microneedles could facilitate drug delivery to the eye and enable significant dose sparing [34][35]

In 2006, Prausnitz et al., investigated for the first time the capability of using coated microneedles to deliver both small molecules (pilocarpine hydrochloride and sodium fluorescein) and macromolecules (bovine serum albumin and plasmid DNA) to rabbit eyes *in vivo* [36]. In this design, a single stainless-steel microneedle with a height of 500-750 μm, a width of 200 × 50 μm and a tip angle of 55° was examined for intraocular drug delivery. As shown in *in vivo* studies, after the insertion of sodium fluorescein-coated microneedles, a drug depot could be formed in the cornea, followed by continuous drug release for hours and resulting in 60-fold higher bioavailability than its topical administration. Consistently, microneedles showed a significant 45-fold increase in the delivery of pilocarpine hydrochloride when compared to topical application.

There are however several limitations to coated microneedle usage in ocular applications that need to be addressed. Most notably, coated microneedles can only load drug molecules onto the available surface area of the needles, limiting their loading capacity [34]. This may therefore warrant the need for frequent administration of these platforms and hence not provide

any further benefit in terms of sustained release in comparison to eye drops, for example. Moreover, poor distribution of coating or choice of material may result in variable drug release rates leading to suboptimal treatment of chronic ocular diseases. Poor repeatability regarding insertion of solid microneedles has also been shown in literature, since the coating process reduces needle sharpness, ultimately decreasing insertion and delivery efficiency [35].

2.3. Hollow microneedles

Similar to conventional hypodermic needles, hollow microneedles enable the injection of liquid formulations through a central bore of a needle, which is commonly fabricated from materials such as stainless steel, silicon and glass. The needle sizes of hollow microneedles, typically ranging from 27 to 35 gauge in outer diameter and starting from 150 µm in length, are significantly smaller than those of conventional hypodermic needles (2 mm or more in length, 26-30 gauge) [37][38]. Therefore, compared to traditional hypodermic needles, hollow microneedles can deliver therapeutic molecules in a more localised and minimally invasive manner to the eye with the potential to improve patient acceptability.

As well as the needle dimensions, the delivery of the formulation can also be tailored. For example, the rate of drug infusion can be controlled as the drug solution is delivered in a pressure-driven fashion [39][40]. Factors such as the injectability of formulation through hollow microneedles can be improved by increasing the dimension of the microneedle bore [39][40]

Previously, Thakur et al., investigated the hybrid delivery system of a single hollow microneedle and thermoresponsive implants to provide long-term ocular drug delivery in a minimally invasive manner [41]. In this study, hollow microneedles with heights of 400, 500 and 600µm were made from hypodermic needles (26, 29 and 30 gauge) and examined for their insertion ability into the *ex vivo* rabbit sclera by optical coherence tomography. After insertion, the sodium fluorescein-loaded thermoresponsive implants were successfully localized in the sclera and sustained the release of the payload over 24 hours. This suggests that the introduction of hollow microneedles has the potential to eliminate the requirement for surgical administration of implants, thereby reducing the risk of surgical complications and improving patient compliance.

Although hollow microneedles have been shown to be effective in facilitating ocular drug delivery, their disadvantages cannot be ignored. Due to the material and small dimensions of hollow microneedles, they are brittle and associated with the risk of blockage and breaking. Furthermore, the fabrication of hollow microneedles, such as deep-reactive ion etching, is more complex and costly in comparison to dissolving microneedles, for example [42][43].

2.4. Bio-inspired microneedles

Bioinspired microneedles, which mimic natural biological structures, have also been designed to improve the efficiency of microneedle administration. Such designs have been synthesized *via* two-photon polymerization to enhance the adhesion of microneedles to the ocular surface as two-photon polymerization capable of printing nanoscale objects with high precision and improved geometry, with a reduced cost of production. For example, Han et al., prepared 4D printed microneedles using poly(ethylene glycol) diacrylate 250 and phenyl bis (2,4,6-trimethylbenzoyl) phosphine oxide as a photoinitiator and Sudan I as a photoabsorber. The bioinspired barbs on the microneedles lead to 18 times higher adhesion of microneedles to the

tissue compared to non-barbed microneedles in *ex vivo* studies using chicken skin (Figure 6b) [44]. This highlights the advantages of such needle designs created using biological references, such as improved needle insertion and retention.

2.5. Combination approaches with microneedles to enhance ocular delivery.

As one of the main static barriers of the eye, the sclera is a matrix embedded with a network of collagen fibers arranged in a random fashion and serves as a barrier to the permeation of drug molecules. Along with the sclera, the choroid-Bruch's membrane and the presence of pigments such as melanin pose remarkable static barriers for the entry of large molecules into the vitreous humor or the site of action.

Many permeation enhancers have been researched to increase the permeation of drugs across tissues [45]. The major disadvantage of these kinds of molecules is that they are irritant to ocular tissues, and biocompatibility and ocular safety remain an issue. Physical parameters such as sonication or iontophoresis can enhance the permeation of drugs across the sclera [46].

Sonication works on the principle of cavitation and vibration. The vibrations created during sonication could enhance/speed up the dissolution of polymeric microneedles. Hence, it assists in faster permeation of therapeutics across the sclera. Similarly, iontophoresis enhances the electrostatic force-driven diffusion of molecules. The electrical charge on the molecules plays an important role in enhancing/managing the flow of molecules across the barrier. Iontophoresis works by enhancing the penetration of ionized compounds across the sclera in the presence of electric charge. Using these methods coupled with microneedles would enhance the penetration of macromolecules such as proteins, oligopeptides and peptides.

Iontophoresis and sonication-assisted microneedles have been found to enhance the permeation of molecules across the sclera under *ex vivo* conditions (Figure 6c) [46]. In a study by Bok et al., the sonication of positively charged rhodamine B was found to be 25 times higher in the presence of 1.7 MHz ultrasound and 10 V AC current. Sonication predominantly enhances the passive diffusion of molecules as opposed to iontophoresis. Iontophoresis enhances the active diffusion of oppositely charged molecules depending on the electric current, and the diffusion of molecules can be managed by altering the electric field. Iontophoresis can also enhance the permeation of proteins that have higher affinity for pigments present in the eye. In a study by Tratta et al., cytochrome C was used as a model protein, and the effect of iontophoresis on the binding affinity and permeability of cytochrome c across the sclera and choroid-Bruch's membrane was studied *in vitro*. Iontophoresis enhanced the permeability of cytochrome C nearly 10 times in the presence of a 2.9 mA/cm^2 current. Moreover, the predominant mechanism of cytochrome c permeation was found to be electrorepulsion, and delivery of large molecular weight proteins can be enhanced by combining iontophoresis with microneedles [47]. Iontophoresis has been found to enhance the delivery of nanoparticles (20 nm) in the suprachoroidal space by more than 30% in *ex vivo* porcine eyes (Figure 6c). Application of 0.14 mA of current resulted in nearly 30% of nanoparticles to the posterior of the eye. The interesting finding in the article suggests that the migration of particles increased with increasing applied voltage for up to 3 mins; however, no change in the nanoparticle distribution was observed up to the application of iontophoresis for 5 mins [48].
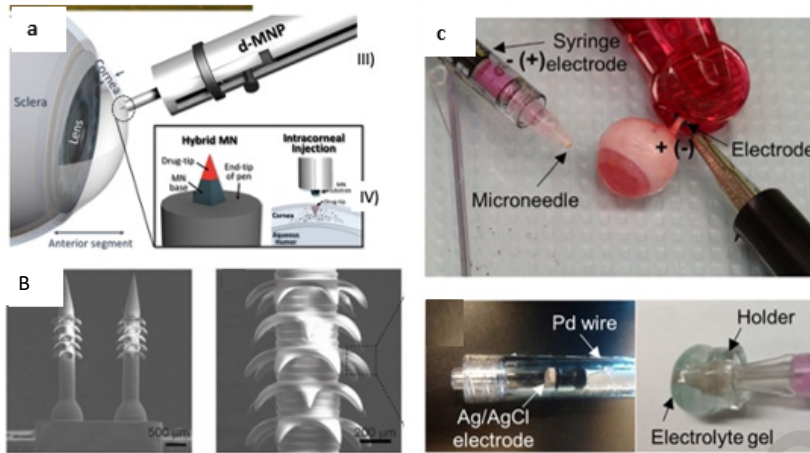
Figure 6: (a) Intracorneal detachable microneedles, (b) 4D printed bioinspired microneedles with greater tissue adhesion [44], and (c) iontophoresis-assisted suprachoroidal drug delivery of nanoparticles (20 nm) using hollow microneedles [48].

Regardless of the type of microneedle selected, it is important that the platform is fully characterised to gain an understanding of the initial safety and compatibility aspects, for example. The following section highlights typical lab-scale methods of microneedle characterisation, which can be applied to all microneedle types discussed thus far.

3. Characterization techniques of ocular microneedles

As previously outlined, it is paramount that the microneedle platform is deemed safe, meaning it can withstand the application force required for efficient insertion and removal without breakage or bending. To determine the safety and performance of these platforms, a number of characterization techniques have been employed in recent years.

Characterization techniques regarding microneedle applications can be classified mainly into two categories: those that concern the characterization of the microneedle platform itself (e.g., fracture force) and those that concern characterization of microneedle insertion efficiency (e.g., the profile and depth of the pore created). Traditional characterization techniques of microneedles are summarized in Figure 7, with their respective outputs and limitations summarized in Table 1.

It must be noted, however, that there is currently a need to establish standardized tests for microneedle characterization to promote the clinical translation of these platforms, which has been somewhat limited thus far [49]. Furthermore, this would enable better comparison between microneedle performance, both at a laboratory scale and clinical scale. This section will discuss various techniques that have been reported in literature to characterize microneedles, with a specific focus on how these techniques can be applied to ocular microneedle applications.
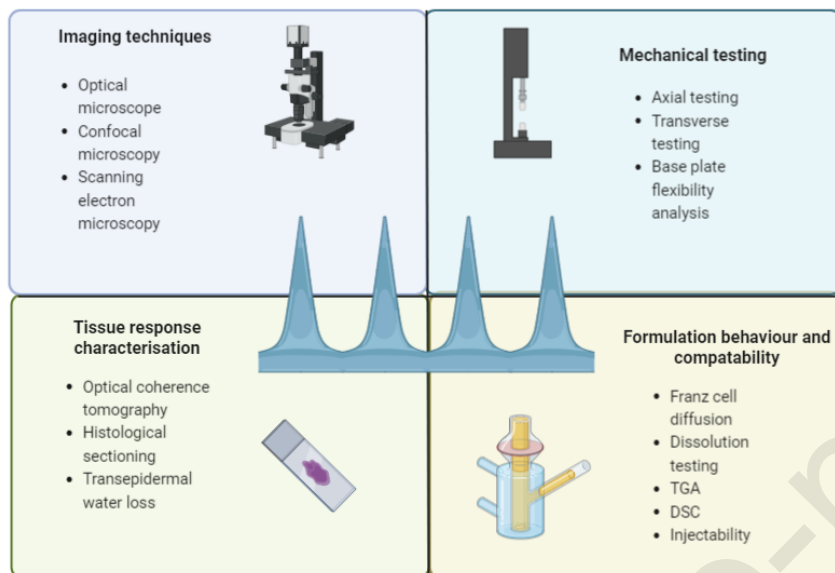
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Figure 7. Schematic diagram of key microneedle characterization techniques.

3.1 Mechanical properties

When first designing the microneedle platform, critical dimensions will be established prior to fabrication, such as the needle height, base width and interspacing. When first designing the microneedle platform, critical dimensions will be established, such as for the needle height, base width and interspacing, prior to fabrication. Since the main aim of this review is to focus on the potential microneedles to be applied to ocular applications, the traditional fabrication techniques of microneedles have not been discussed in depth within this review. As this topic has already been covered extensively in literature, therefore we refer readers to Microneedles book by Kevin Ita for further in detail information on microneedle fabrication techniques [50].

Following microneedle fabrication, imaging techniques such as optical microscopy and scanning electron microscopy are commonly employed to check the accuracy of the final dimensions prior to mechanical testing. Additionally, these techniques can be used to confirm that the microneedle surfaces are smooth and that no drug aggregation or deposits have occurred on the surface.

Once the expected dimensions have been confirmed, mechanical tests are employed to establish how the microneedle platform responds to stresses, which is determined by properties such as fracture force and Young's modulus. In the case of dissolving microneedles, for example, a range of factors, including polymer type, drug concentration and moisture content, all influence the resultant mechanical properties [51]. Despite being commonly researched for ocular applications, there is currently a lack of focus on the mechanical properties of metallic hollow microneedles; thus, this requires further analysis.

14

One of the most basic tests to characterize the mechanical properties is through axial testing, or compression testing, whereby a texture analyzer setup equipped with a load cell is used to apply a predefined force perpendicular to the baseplate at a controlled speed [52]. Following testing, a force displacement curve is created, from which material physical properties, like Young's modulus, can be calculated and the microneedle platform can be examined for any sign of failure.

Furthermore, using the same texture analyzer setup outlined above, a force can also be applied in a direction parallel to the base plate to simulate bending of the needles under insertion. Upon insertion, microneedles naturally and unavoidably experience a degree of bending due to the elasticity of the tissue as well as its nonuniform surface profile [52]. It must therefore be established what force the needles can experience in the transverse plane before failure occurs.

Ultimately, mechanical testing is conducted to establish if there is an acceptable ratio between the fracture force and the insertion force of the microneedle (i.e., the 'margin of safety') in both axial and transverse planes. While the ratio should be as high as possible, a minimum safety margin of greater than one is crucial [53]. It has been previously demonstrated that microneedle design parameters, such as needle height and formulation/material, affect the safety margin ratio, with decreasing needle heights shown to increase the fracture force of the needles [54]. Recently, Alimardani et al. studied the effect of drug loading on the mechanical properties of PNIPAAm51-b-PGA10-based dissolving microneedles for the intrascleral delivery of dexamethasone using *in situ* forming nanomicelles. Through compression testing, they found the height reduction of drug-loaded microneedles to decrease from approximately 40 to 25% as the polymer content was increased from 15 to 30% w/v (Figure 8a) [55].

Despite the frequency of their use, there are still some limitations associated with these tests, namely, that the *in vitro* test conditions are not physiologically accurate. While stresses experienced during microneedle insertion in a clinical setting are typically distributed across the whole array as a result of tissue elasticity, mechanical testing cannot accurately replicate this since the solid surface in contact with the needle during testing means stresses are concentrated at the needle tip under these test conditions [51].

Additionally, mechanical characterization should not be reserved only for the needles themselves. The strength of the base plate, as well as its flexibility, also plays a key role in the efficacy and safety of these platforms.

The baseplate needs to possess sufficient flexibility to adapt to the natural heterogeneous surface profile of the tissue, particularly in the case of ocular applications; therefore, material selection is a factor in determining the rigidity of the base plate.

3.2 Insertion depth

Determining the depth of insertion of the microneedle into the target tissue is crucial to understanding the efficiency of the microneedles as a drug delivery platform. While complete insertion of the needles is typically not achievable [56], it is desirable to maximize the percentage of the total needle height that is inserted into the tissue. This will maximize drug deposition into the target tissue and thus improve the potential for positive therapeutic outcomes.

In vitro insertion studies are usually conducted to characterize the depth of insertion into excised tissue samples. Recently, Wu et al. conducted insertion studies into porcine sclera,

whereby 3 x 3 arrays of PVA/PVP microneedles were inserted at a predefined force of 3 N. After a holding period of 30 seconds, 76% of the needle height (569 ± 15.31 μm) was observed to be inserted into the sclera using optical coherence tomography (OCT). OCT is one of the most commonly employed techniques used to characterize the insertion depth of microneedles into tissue since it provides imaging at depths up to 2000 μm and is nondestructive [57]. While OCT typically has poorer resolution than confocal imaging (up to 4 and 1 μm, respectively), it is capable of achieving larger imaging depths than confocal imaging (200 μm) [57].

Histological sectioning is often used alongside OCT imaging to confirm the insertion depth of the needles and provide a more defined profile of the pore created by the microneedles. Observation of the pore using histological sectioning methods is often aided by the administration of dye, typically fluorescent dyes, such as sulforhodamine B. Despite this, however, the process of histological staining and sectioning can prove time-consuming. Previously, Jiang et al. demonstrated the distribution of sulforhodamine B in the human sclera *ex vivo* following application of a single hollow microneedle (Figure 8b) [58].

Alternative methods for insertion testing, which do not require the use of biological tissue, have also been investigated. For transdermal microneedle applications, Larreneta et al., developed a substitute model for porcine skin typically used in insertion testing by folding commercially available Parafilm® to a similar thickness of the skin [59]. While such models would provide an inexpensive and easy alternative to tissue samples to quickly prescreen microneedles, a similar model is currently lacking for ocular microneedle testing.

3.3 Pore closure

Transepidermal water loss (TEWL), which provides a quantitative measurement of tissue disruption by measuring the rate of water vapor density around the insertion site [60], is a noninvasive and popular method for measuring pore closure following microneedle administration for transdermal applications. However, such experiments must be conducted under carefully controlled conditions since factors such as temperature and room humidity will affect the results obtained [61]. One of the methods to study the pore closure following microneedle penetration is measurement of Transepithelial/transendothelial electrical resistance (TEER). TEER is commonly used to measure the integrity and permeability of cellular monolayers. However, it is also an attractive tool for quantifying the pore closure of microneedles in skin or ocular tissue. The Ohmic resistance of tissue is inversely proportional to pore formation hence the permeation of tissue could be quantified by estimation of TEER [62].

3.4 Formulation compatibility with microneedle

The drug content within the dissolving microneedles must first be determined prior to any *in vitro* release studies. This requires careful separation of the needles and the base plate, after which the needles can be dissolved, typically in PBS, and analyzed by suitable quantification methods, such as high-performance liquid chromatography.

For dissolving microneedles, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) are also often employed to assess the physical and thermal stability of microneedles, respectively. This can be conducted on the initial polymers and drug, as well as the final formulation used for microneedle fabrication. Arshad et al. conducted DSC and TGA following the fabrication of a PVA/sorbitol-based dissolving microneedle array loaded with heparin-sodium. In DSC, they found incorporation of sorbitol to impart a plasticizing effect on

the PVA polymer, which would ultimately reduce the brittleness of the formulation, while TGA demonstrated stability of the formulation between 65 – 240°C [63]. FTIR analysis is also commonly conducted after drug loading to ensure no drug-polymer interactions [64].

Dissolving microneedles are also subjected to dissolution testing since the rate of dissolution directly impacts the release rate of the loaded drug. Dissolution should occur rapidly in ocular tissues to make these platforms patient friendly. However, this process should occur at a controlled rate and ensure that complete needle insertion is possible before any dissolution occurs, which otherwise will negatively impact the mechanical integrity of the needles.

These studies are typically conducted either *ex vivo* or in vivo, whereby the microneedle array, once administered to the tissue, is removed at predefined timepoints for observation of microneedle integrity using basic imaging techniques such as optical microscopy [65]Thakur et al., conducted dissolution testing on PVP-based dissolving microneedles in cornea and sclera samples *ex vivo*. Percentage height reduction was calculated at several defined time points, with the optimal formulation demonstrating complete needle dissolution within two minutes in scleral tissue (Figure 8c) [45].
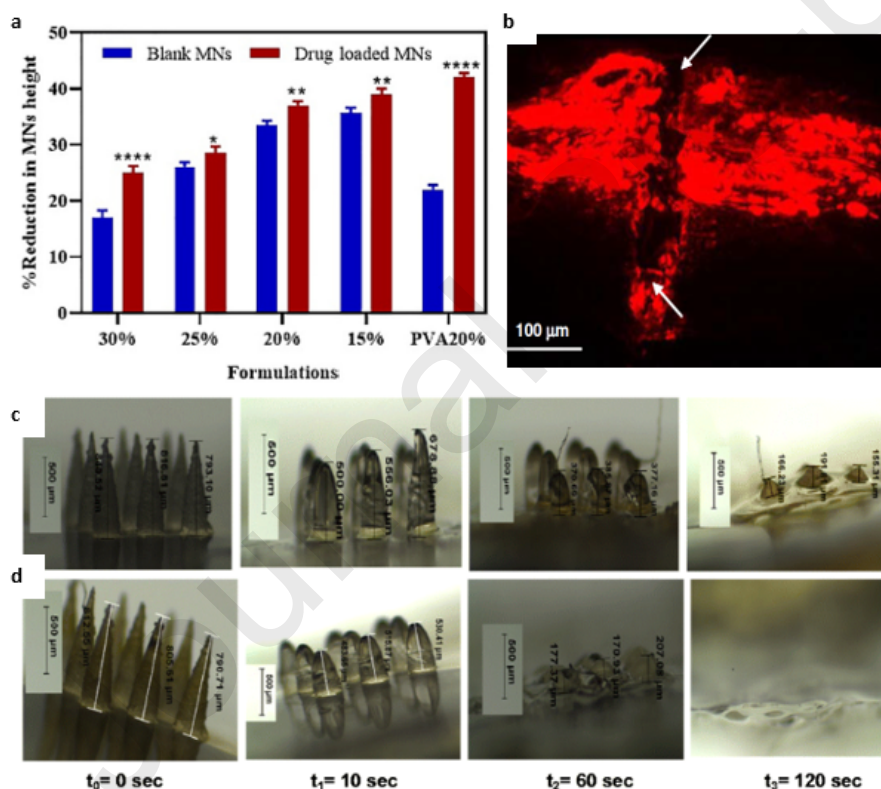

Figure 8. (a) Percentage height reduction of PNIPAAm51-b-PGA10-based dissolving microneedles as a result of polymeric content [55] (b) Histological section of sclera following injection of sulforhodamine B using a single

hollow microneedle [58], (c, d) Digital images of fluorescein isothiocyanate–dextran-loaded PVP microneedle arrays in the (d) cornea and (e) sclera during dissolution testing [51].

Finally, in the case of hollow microneedles, syringeability and injectability must also be determined. As the formulation must pass through the central bore present in the microneedles as it is infused into the target tissue, the force required to pass it through this bore must be determined.

As recommended by the guidance of the Royal College of Ophthalmologists, the use of smaller hypodermic needles (typically 27 or 30 gauge) is preferred for ocular injections to reduce pain on administration [66]. These needles have small internal diameters (~ <0.4 mm), and as a result, the viscosity of the formulation suitable for administration is limited to avoid clogging of the needle or excessive force required for infusion. Similarly, care must be taken with particulate systems delivered using hollow microneedles, as aggregation of particles could also lead to needle clogging issues.

To avoid such issues, the use of *in situ* forming hydrogels has been investigated. In these systems, the drug formulation exists as a solution that enables good syringeability and injectability, but upon administration to the target tissue, the formulation forms a depot to enable sustained drug release. Thakur et al., previously demonstrated the potential for intrascleral delivery of drug-loaded thermoresponsive poloxamer formulations at low forces [25].

3.5 Permeability studies

The Franz-cell diffusion setup is another technique commonly used within microneedle characterization to measure drug permeation through biological tissue following microneedle application. In this setup, the tissue sample is placed between two compartments, the donor and receptor, which contain suitable testing medium, typically phosphate buffered saline for tissue. At predefined timepoints, samples of the medium are then removed for quantitative drug content analysis and replaced with fresh medium. Furthermore, following completion of the test, the tissue sample can be homogenized to quantify the drug remaining in the tissue sample, enabling comparison between the amount of drug permeated versus remaining in the tissue. This setup enables comparison between the microneedle-treated tissue samples and those that are treated with a control group, for example, topical application of the same drug, to test the efficacy of microneedles as a drug delivery platform. A few minor limitations of the Franz-cell setup include the large tissue sample size required for analysis, which could be difficult to generate for the eye, as well as the unavoidable introduction of gas, which will affect diffusion, during each time point for sampling [67].

The absence of physiological functions of the eye, such as intraocular pressure, tear film secretion, and other factors, can limit the translational value of the results obtained from the Franz-cell diffusion setup. Furthermore, the variations in the scleral tissue properties over time such as the porosity, swelling, collagen fiber contraction, loss in tissue integrity are not yet well understood – potentially leading to missed interpretations of the permeation data. Furthermore, the static and flat tissue required for use of this setup is not representative of the curved viable tissue in *in vivo* conditions. Therefore, the development of *ex vivo* setups which enable replication of *in vivo* conditions, such as the accurate shape and perfusion of the eye, are highly

18

desirable to gain better understanding of transscleral diffusion and importantly draw correlations between *in vitro*, *ex vivo* and *in vivo* models [61].

Both formulation compatibility and permeability will undoubtedly be influenced by the selected therapeutic itself. Since research into ocular microneedles began, a range of therapeutics have been delivered through these platforms, ranging from small molecules to biologics, for example. The following section discusses such studies.

Table 1. Table highlighting the key characterization techniques for microneedles and their associated limitations

Type of test	Type of microneedle	Output information	Challenges/drawbacks	References
Scanning Electron Microscopy	Dissolving, hollow and solid	• Microneedle dimensions. • Confirmation of no drug aggregation on microneedle surface	• Limited sample size. • Sample must be stable within a vacuum. • Potential occurrence of artifacts.	[68]
Axial/transverse mechanical testing	Dissolving, hollow and solid	• Mechanical properties, i.e., fracture force and young's modulus. • Margin of safety	• Test conditions are not physiologically accurate. • Stress distributions experienced in the arrays are not representative of physiological conditions.	[69]
Base plate flexibility testing	Dissolving	• Mechanical properties of the base plate • Flexibility of the base plate	• Test conditions are not physiologically accurate.	[69] [70]
Insertion depth testing	Dissolving, hollow and solid	• Force required for needle insertion.	• Difficult to mimic disease states (e.g., increased stiffness of tissue).	
Optical Coherence Tomography	Dissolving, hollow and solid	• Depth of needle insertion into the tissue.	• 2D representation of tissue. • Unable to synchronize axial scanning and focus control. • Poorer resolution compared to confocal imaging.	[71] [57].

			• Time consuming.	
Histological sectioning	Dissolving, hollow and solid	• Depth of needle insertion into the tissue. • Dye distribution in tissue following administration.		
Transepidermal water loss	Dissolving, hollow and solid	• Time taken for pore closure.	• Affected by environmental conditions e.g., temperature and humidity.	[57].
Thermogravimetric analysis	Dissolving	• Thermal stability of microneedles.	• Influenced by environmental conditions. • Slow heating rates can affect the achievement of isothermal conditions.	[72]
Differential scanning calorimetry	Dissolving	• Physical stability of microneedles.	• Influenced by environmental conditions. • Destructive method.	
Fourier transfer infrared spectroscopy	Dissolving	• Identification of drug-polymer interactions.	• Potential for interference between multiple absorbing components.	[73]
Dissolution testing	Dissolving	• Time taken for microneedles to dissolve following administration.	• Difficult to recreate physiological conditions.	
Franz cell diffusion	Dissolving, hollow and solid	• Rate of drug permeation through the tissue following administration.	• Large sample size needed. • Introduction and interference from gas.	[67].

4. Therapeutic molecules and routes of administration for ocular microneedle platforms

As outlined, the application of microneedles for ocular therapy has grown in popularity. The underlying cause and location of the ocular disease determine the type of medication most suitable for the application. There are typically three types of ocular pharmacological agents, namely, anti-VEGF agents, anti-inflammatory agents, and anti-glaucoma agents, which can be used to treat neovascularization, inflammation and glaucoma. Microneedles have been applied as an alternate novel delivery system to facilitate the delivery of these ocular pharmacological agents to treat ocular diseases.

The intended treatment also dictates what type of microneedle is suitable for delivery. For example, dissolving microneedles demonstrate promising suitability for eye diseases since they can be applied in a similar manner to contact lenses. Due to the familiarity of contact lens usage, this is likely to improve patient acceptability of these microneedle platforms for anterior segment disease treatments. Meanwhile, hollow and solid microneedles can be used to target posterior segment diseases by administration to the sclera and suprachoroidal space and will require much more precise administration procedures, which will be conducted in a clinical setting. The suitability of the microneedle type for the intended application can be demonstrated by the delivery of model drugs.

For example, previous studies conducted by Thakur et al., demonstrated the feasibility of delivering both small molecules (fluorescence sodium and amphotericin) and macromolecules (fluorescein isothiocyanate (FITC)-dextran with molecular weights of 70 kDa and 150 kDa) to the posterior segment of the eye [31][74]. In this design, dissolving microneedles with 9 conical needles measuring 800 μm in height and 300 μm in width were fabricated from PVP of various molecular weights. Several *in vitro* (mechanical, insertion and dissolution tests) and *ex vivo* studies (permeation and distribution tests) were performed to characterize the capability of the developed microneedle to facilitate the delivery of small molecules and macromolecules to the back of the eye. They illustrated that PVP microneedles were strong and sharp enough to penetrate the corneal and scleral barriers. There was a 10-fold increase in macromolecules delivered using dissolving microneedles compared to topical delivery. This group also investigated the delivery of ovalbumin-encapsulated PLGA nanoparticles using dissolving microneedles into the sclera to achieve long-term release of ovalbumin to the target tissue in the posterior segment of the eye for up to 2 months [56]. In this study, a bilayered microneedle was created by concentrating therapeutic molecules exclusively in the needle part of the microneedle to reduce cargo waste (Figure 9a), resulting in improved drug bioavailability. The developed FITC-ovalbumin nanoparticle-loaded microneedles were successfully inserted into the sclera (Figure 9b). Images from multiphoton microscopy (Figure 9c) revealed that the introduction of microneedles could significantly improve the delivery of macromolecules and nanoparticle to the back of the eye, thereby achieving increased delivery efficiency and higher therapeutic efficacy of retinal diseases.
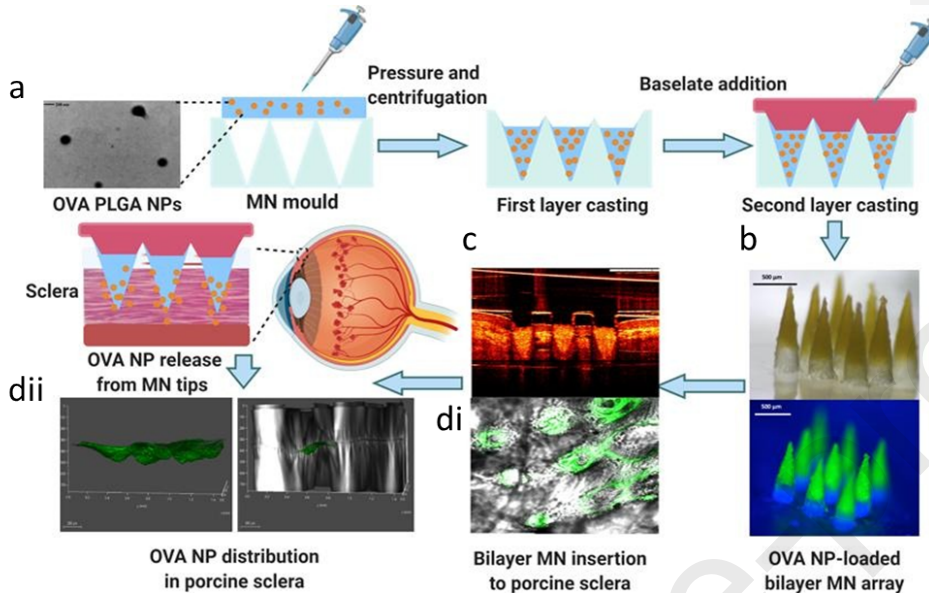
22

Figure 9. a) Schematic representation of the preparation of OVA NP-loaded bilayer microneedle arrays. b).Fluorescence microscopic image of FITC-OVA NP-loaded bilayer microneedles with FITC-OVA NP (green color) in the needle part and the polymeric matrix (blue color) in the bottom part. c) The OCT image of bilayer microneedle array following insertion into the porcine scleral tissue(scale bar =1 mm). di) The multiphoton microscopic images of FITC-OVA penetration in the porcine sclera and FITC-OVA were green labeled; surface image of the sclera following the application of FITC-OVA NP-loaded microneedles. dii) 3D visualizations of FITC-OVA and FITC-OVA NP penetration in porcine sclera treated FITC-OVA nanoparticle-loaded microneedles after 24 h of application [75].

4.1 Microneedles for anti-VEGF agent delivery

Abnormal neovascular diseases such as AMD, diabetic retinopathy and choroidal neovascularization are the leading causes of irreversible blindness. All of these conditions are caused partly by a protein called VEGF, the overexpression of which not only promotes the generation of new, fragile vessels but also enhances the permeability of existing vessels, leading to blockages, leakage and hemorrhage [76]. Anti-VEGF agents are the first choice for alleviating angiogenic pathologies. Most anti-VEGF agents work by suppressing the binding of VEGF-A, a key regulator of angiogenesis and permeability of blood vessels to VEGF receptors (VEGFR-1 and VEGFR-2) [77]. Although overexpression of VEGF can induce ocular neovascular diseases, it is undeniable that VEGF is essential for the rest of the body, especially for the growth of new blood vessels. Excessive anti-VEGF agents in the systemic circulation may increase the risk of cardiovascular diseases such as hypotension, reduced heart pulse rate, heart attacks and strokes [78]. Thus, localized and targeted administration of anti-VEGF agents is desirable for ocular disease treatment. Microneedles are considered an optimal choice for the administration of anti-VEGF agents, as they can locate therapeutic molecules directly in the eye in a minimally invasive manner, thereby reducing the risk of systemic adverse effects and improving patient compliance.

23

Lee et al., developed tower microneedles and proved the highly efficient and minimally invasive properties of tower microneedles in delivering anti-VEGF agents to the eye [79]. The tower microneedle, a type of hollow microneedle with a length of 5 mm and a diameter of 120 μm, which is approximately half of the 30 G hypodermic needle, was developed in this investigation. The disease model was generated by inducing neovascularization in mice with laser irradiation. The developed tower microneedle was shown to be able to induce comparable degrees of anti-angiogenesis in the retina as the 30 G hypodermic needle. Furthermore, by using sodium fluorescein to confirm the size of the bleb in the treated eyes, the tower microneedles were demonstrated to be less invasive with minimal bleb formation.

Kim et al., stated that compared with subconjunctival and topical administration, coated microneedles could target drug delivery to the site of neovascularization (corneal stroma), enabling effective treatment of corneal neovascularization [80]. Specifically, the disease model was established by placing a silk suture in the cornea of healthy New Zealand rabbits, resulting in corneal neovascularization associated with minor traumatic injury. Due to the tight-conjugated corneal epithelial cells, topical administration of proteins exhibits limited effectiveness in treating corneal neovascularization and required 52500 μg of bevacizumab to show initial therapy of corneal neovascularization. Through topical administration, only a limited amount of anti-VEGF agents (< 5%) can be delivered to the target site, while the majority is cleared by the subconjunctival–episcleral blood and lymph vessel flow and the choriocapillaris blood flow and then absorbed into systemic circulation *via* the highly vascularised nasolacrimal duct, which can lead to adverse systemic effects [8,9]. Although subconjunctival injection could provide more efficient delivery of macromolecules, 2500 μg bevacizumab (463-fold higher) was required to show the same therapeutic capability as 4.4 μg bevacizumab dosed directly in the cornea *via* coated microneedles. The necessity of such high doses is likely to be toxic or cause intraocular side effects such as thinning or erosive changes to the conjunctiva and sclera [10,11]. By bypassing the ocular barrier and localizing therapeutic molecules precisely into the site of action, coated microneedles enable significant dose sparing and improved therapeutic efficacy, thereby reducing adverse effects and complications associated with high doses required by conventional administration routes.

For posterior segment applications, Patel et al., demonstrated the feasibility of using hollow microneedles targeted to the suprachoroidal space to deliver bevacizumab to the posterior segment of rabbit eyes. As the suprachoroidal space is located between the sclera and choroid and adjacent to the retina, injected materials can be efficiently delivered to the posterior segment of the eye, thereby reducing dosing requirements. In contrast, intravitreal injection at the same dose resulted in lower drug levels in the back of the eye because of limited selectivity for posterior versus anterior segment tissues [85].

Coated microneedles have also shown promising results in facilitating drug delivery to the posterior segment of the eye. Kim et al., stated that compared with subconjunctival and topical administration, coated microneedles could considerably improve the delivery efficiency of bevacizumab to the back of the eye [39]. Specifically, coated microneedles loaded with 4.4 μg of bevacizumab showed similar therapeutic effects in treating choroidal neovascularization as 2,500 μg and 52,500 μg of bevacizumab delivered *via* subconjunctival injection and eye drops, respectively.

4.2 Microneedles for anti-inflammatory agent delivery

24

Ophthalmic anti-inflammatory medications are mainly divided into corticosteroids and nonsteroidal anti-inflammatory agents. Due to their intensive anti-inflammatory effects and potential anti-angiogenesis function, corticosteroids (e.g., dexamethasone, triamcinolone acetonide and fluocinolone acetonide) are considered the mainstay treatment for ocular inflammation [86]. However, it is undeniable that the adverse effects of corticosteroids, such as elevated intraocular pressure and cataract formation, are of great concern.

Corticosteroids can be delivered to the eye by various routes of administration, the choice of which depends primarily on the site of treatment. Topical administration of corticosteroids *via* eye drops or ointments is preferred when targeting inflammation of the ocular surface and anterior segment (e.g., conjunctivitis, anterior uveitis and scleritis). In general, frequent administration is required to maintain the therapeutic level of corticosteroids during the first 24-48 hours, while after the inflammation is controlled, the frequency of administration can be reduced. However, it is noteworthy that due to the requirement of frequent application, topical and systemic corticosteroid administration has been linked to intraocular pressure elevation, slow wound healing and increased risk of glaucoma and cataracts [87]. Shields et al. demonstrated that the one-month application of corticosteroids *via* eye drops could significantly increase the intraocular pressure of 5% of patients to > 16 mmHg and 30% of patients to 6-16 mmHg [88]. Thus, careful follow-up after topical administration of corticosteroids, such as topical medication or even surgery, is required to normalize intraocular pressure [89]. For the treatment of posterior ocular inflammatory diseases, intravitreal injection of corticosteroids is considered the mainstream therapy. However, because of the high invasiveness of intravitreal injection *via* hypodermic needles, to reduce dose frequency and improve patient compliance, a high dose of corticosteroid needs to be injected *via* a single intravitreal injection, which can lead to severe adverse effects. For example, clinically, a single intravitreal injection contains high doses of triamcinolone acetonide (4-25 mg), which potentially results in elevated intraocular pressure and, in turn, leads to secondary ocular hypertension and secondary chronic open-angle glaucoma [15,16]. Accordingly, a less invasive, targeted and convenient administration method of anti-inflammatory agents is highly desirable. Microneedles can bypass ocular barriers and localize corticosteroids to target sites in the eye, making them a safe and effective administration route for anti-inflammatory agents.

A typical corticosteroid, triamcinolone acetonide, was dosed by hollow microneedles to treat acute posterior uveitis. In detail, a single 33 G hollow microneedle with a height of 850 μm was developed to dose triamcinolone acetonide into the SCS suprachoroidal space and compared with intravitreal injection *via* a hypodermic needle (27 G) for therapeutic efficacy as well as the risk of adverse effects by determining ocular inflammatory scores and intraocular pressure. The results showed that 0.2 mg of triamcinolone acetonide dosed to the suprachoroidal space *via* microneedles could generate an anti-inflammatory effect comparable to 2 mg of triamcinolone acetonide administered *via* intravitreal injection. This 10-fold reduction in effective dose suggests a significant role for microneedles in targeting drug delivery to the choroid and retina, thus lowering the risk of adverse effects, increased intraocular pressure and drug toxicity of triamcinolone acetonide [92]. Moreover, Roy et al., demonstrated the use of dissolving microneedle (5 x 5 needles/array, 550 μm length and 300 μm height) to deliver triamcinolone acetonide to the back of the eye[93]. Compared with intravitreal injection, transscleral microneedles provided better safety outcomes and significantly higher triamcinolone acetonide deposition in the sclera. As a result, microneedles are thought to be an appealing alternative to hypodermic needles for minimally invasive and targeted intraocular delivery of corticosteroids [93].

In addition to anti-inflammatory effects, corticosteroids can also be utilized to treat ocular neovascular diseases, as they can decrease vascular permeability, limit VEGF expression and endothelial proliferation, and induce vasoconstrictive effects [19-22]. Than et al., developed and characterized self-implantable double-layered dissolving microneedles to treat corneal neovascularization. In this design, double-layered microneedles were prepared with hyaluronic acid as the inner core and crosslinked methacrylated hyaluronic acid as the outer layer to provide arrays with rapid dissolution, adequate stiffness and biphasic drug release kinetics. To achieve rapid release of anti-inflammatory agents followed by long-term action of anti-VEGF agents, a model antiangiogenic monoclonal antibody (DC101) and an anti-inflammatory agent (diclofenac) were loaded in the outer layer and inner core of microneedles, respectively. Furthermore, by inducing the disease model in healthy mice, it was demonstrated that the combined administration of anti-VEGF and anti-inflammatory agents could provide synergistic effects for treating corneal neovascularization in a user-friendly and efficient manner [96].

4.3   Microneedles for antibiotic delivery

While diseases affecting the anterior segment of the eye are typically less sight-threatening and easier to treat due to the accessibility of the tissues, they are very common. Dissolving microneedles in particular have been widely applied for the delivery of therapeutic molecules to the anterior segment of the eye, thereby treating anterior diseases such as infections and glaucoma.

In 2017, Bhatnagar et al., used PVP/PVA-based dissolving microneedles with a 6 × 6 needle array to deliver the model antibiotic besifloxacin to treat bacterial infections in the cornea. The designed besifloxacin-loaded microneedles were proven to be capable of bypassing the barrier of the cornea and efficiently limiting ocular infections with greater concentrations of besifloxacin detected in corneal tissue than in drug solutions. Furthermore, unlike free besifloxacin solution, microneedles can act as a depot in the cornea, thereby prolonging the duration of action and significantly reducing the frequency of topical drug administration and ultimately improving patient acceptability [97].

Similarly, Roy et al., also used dissolving microneedles prepared from a mixture of PVP and PVA to deliver the antibiotic amphotericin B to treat corneal diseases. Amphotericin B was formulated into soy phosphatidylethanolcholine liposomes to increase the delivery efficiency and stability while decreasing toxicity. The developed amphotericin B-loaded microneedles could significantly reduce the *Candida albicans* load in the cornea both in *ex vivo* and rabbit models, thereby effectively alleviating fungal keratitis [98].

Albadr et al., also reported the fabrication of amphotericin B loaded rapid dissolving microneedles for management of intracorneal infections. The rapid dissolving matrix for amphotericin B loading was prepared using mixture of PVP and hyaluronic acid. The multiphoton microscopy suggested successful amphotericin B depot formation after intrascleral administration of microneedles. The direct loading of amphotericin B lead to higher drug lading as well as high mechanical strength as suggested by authors [99]

3.4. Microneedles for antiglaucoma agent delivery

Glaucoma, the second leading cause of irreversible blindness, is caused primarily by a marked increase in intraocular pressure [100]. Approximately 67 million adults worldwide suffer from glaucoma, and the incidence of glaucoma increases with age [101]. Because of their convenience and avoidance of first-pass metabolism, topical drops containing intraocular

pressure -lowering medication are the most commonly used treatment for glaucoma. However, topical drops always result in poor bioavailability (typically < 5%) due to the complicated structure and barriers of the eye as well as rapid drainage caused by gravity or through the nasolacrimal duct [102]. To make matters worse, drug molecules entering the eye are delivered nonspecifically throughout the anterior segment, with only a small portion of the drug achieving its site of therapeutic action. Due to the poorly targeted nature of eye drops, there is significant systemic absorption (up to 80%), causing adverse effects in 8% to 53% of glaucoma patients taking topical antiglaucoma drugs [27,28]. Furthermore, as the proper administration of topical drops requires the correct instillation of the eye drop onto the eyeball and multiple medications per day, it is challenging for many patients, especially elderly patients [105]. Therefore, novel drug delivery systems are required to provide safer, more convenient and effective drug delivery. Microneedles are deemed a promising technique for targeted delivery of antiglaucoma drugs to the eye, significantly improving delivery efficiency and patient compliance. The number of published studies has progressively increased over the years, indicating that microneedles are an attractive carrier for antiglaucoma administration.

Prausnitz et al., investigated the transscleral delivery of anti-glaucoma drugs (sulprostone and brimonidine) by using hollow microneedles. In this study, an individual 33 G stainless steel microneedle measuring 700 to 800 μμm in length was inserted into the sclera and tested for its capability of delivering antiglaucoma drugs to the supraciliary space. Both sulprostone and brimonidine were efficiently and targeted delivered to the site of therapeutic action (ciliary body) using hollow microneedles and generated similar effects with a 100-fold dose reduction compared to topical application. The dose-sparing effect of hollow microneedles can significantly reduce adverse effects at off-target sites and thereby improve patient acceptance [106]. Moreover, this group used the developed hollow microneedles to target delivery of brimonidine-encapsulated microspheres to the supraciliary space [107]. In *in vivo* studies conducted using New Zealand rabbits, the developed hybrid system of microspheres and hollow microneedles was shown to be capable of providing sustained-release treatment of glaucoma for up to one month *via* a single injection. Targeted antiglaucoma agent delivery in this way is well tolerated without side effects and enables long-term drug release *via* a single administration.

Roy et al., manufactured dissolving microneedles composed of PVA and PVP to mimic contact lenses to deliver pilocarpine across the cornea. *Ex vivo* studies in excised porcine eyeballs revealed that, when compared to conventional topical drops, they were able to provide rapid administration with significantly improved flux and availability of pilocarpine in the aqueous humor [108]. Consistently, Khandan et al. also emphasized the significant dose-sparing effect of microneedles in facilitating the delivery of pilocarpine to alleviate glaucoma [109]. Currently, pilocarpine is administered *via* topical drops, and four doses per day are required to effectively reduce intraocular pressure. However, due to the limited bioavailability of eye drops, topical pilocarpine has been associated with numerous intraocular (e.g., irritation of the eye, retinal detachment, headache, and blurry vision) as well as systemic side effects (e.g., nausea, vomiting, and diarrhea) [29,34]. By bypassing the corneal barrier and targeting the delivery of pilocarpine to the ciliary body, microneedles could circumvent the necessity for repeated daily dosing and significantly improve the therapeutic efficacy of pilocarpine, thus reducing the risk of side effects. Overall, microneedles provide an important advance in the targeted delivery of anti-glaucoma drugs to improve intraocular bioavailability and safety.

27

5. Clinical pipeline of microneedles

Even after approximately 25 years of research for application of microneedles in ocular drug delivery, only one microneedle product has been approved for ocular drug delivery applications. In October 2021, Clearside Biomedical secured approval XIPERE®, a single stainless steel hollow microneedle for the delivery of triamcinolone acetonide suspension into the suprachoroidal space to treat DME. The precise and localized delivery into this region is facilitated using the SCS Microinjector® device (Figure 10) and exploits the pressure gradient within the suprachoroidal space to distribute the drug across the entire space (Figure 10).
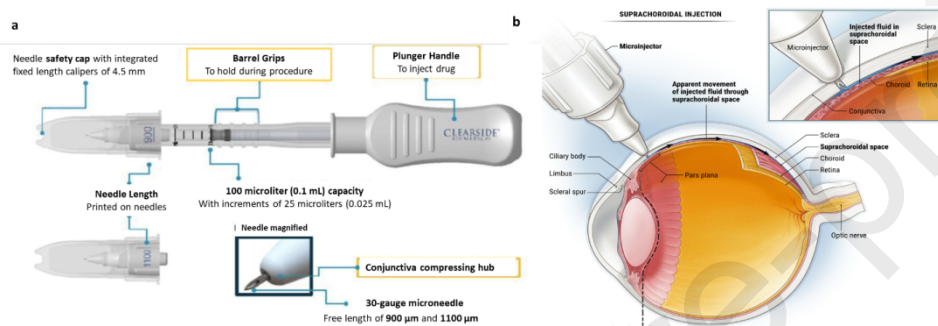

Figure 10: a) Xipere Microinjector® for targeted suprachoroidal drug delivery[112] b) The administration of drug into the SCS, which is situated between the choroid and sclera. During the suprachoroidal injection process, the expanded state of the SCS (represented by the blue area) is observed. Image adapted from Wilner et. Al., 2019 [113]

In a phase 3 randomized trial (PEACHTREE, NCT02595398) by Clearside Biomedical, for suprachoroidal injection of triamcinolone acetonide CLS-TA, improvement in patient vision was assessed. The primary end point at week 24 was stated to be a BCVA (Best Corrected Visual Acuity) improvement of at least 15 ETDRS (Early Treatment of Diabetic Retinopathy Study) letters. The primary end point was reached in 46.9% of patients who were given 4 mg CLS-TA *via* suprachoroidal injection at Day 0 and again at week 12, versus only 15.6% for the sham group. Safety endpoints were also achieved, with just over half of patients (51%) experiencing adverse ocular events (compared to 58% in the sham group). Cystoid ME was the most prevalent adverse event in the sham group, while 12.5% and 11.5% of patients experienced pain and increased intraocular pressure, respectively [114].

Moreover, an observational extension study of PEACHTREE, MAGNOLIA (NCT02952001), demonstrated a median rescue time of 344 days following the original PEACHTREE trial [115]. An another additional Phase 3 safety trial conducted (38 patients, 20 with ME) showed mean intraocular pressure to increase from 13.3 to 15.2 mmHg after 24 weeks, with only 15.8% of patients intraocular pressure fluctuating greater than 10 mmHg at any visit (AZALEA, NCT03097315), showing excellent safety profiles.

These clinical studies followed up from their promising Phase 2 data (DOGWOOD, NCT002255032) in which the efficacy of two doses, 4 mg or 0.8 mg in 100 µl, was assessed. In just over two thirds of patients, an improvement greater than 20% was observed regarding central subfield thickness and a subsequent improvement in patient vision was also observed.

Further early-stage trials by Clearside Biomedical, such as HULK (NCT02949024), assessed changes in suprachoroidal space thickness immediately following administration of the triamcinolone acetonide formulation (40 mg/ml). Despite a significant thickness increase (65.1 μm to 75.1 μm) immediately following administration, it was however confirmed that no significant changes in suprachoroidal space thickness versus untreated eyes were present up to almost 5 months later using OCT [116,117]

The potential benefits of combination therapy with aflibercept were also explored in a Phase 2 trial (TYBEE, NCT03126786). Patients received either a single dose of aflibercept *via* intravitreal injection at four-week intervals up to week 12 (control group), or a single dose of CLS-TA (40 mg/ml) and aflibercept (2 mg/0.05 mL) on day zero, followed by a single dose of aflibercept on week 12 (treatment group). While there was no significant difference between mean BCVA scores, combination therapy reduced the number of treatments required. Nonetheless, similar benefits were established between the two groups, with the potential to reduce the clinical burden associated with DME treatment [116,118]

Similarly, early-stage Phase 1 and 2 trials (TRIESENCE, NCT01789320) were conducted dating back to 2013 for triamcinolone acetonide delivery to suprachoroidal space for non-infectious uveitis. The same dose was utilized as for trails concerning the formulation for DME treatment (40 mg/ml). Minimal fluctuations from baseline intraocular pressure were observed (-0.1 – 1.3 mmHg), indicating the potential safety of this approach. Moreover, following a single dose, BVCA improvements in the range of 8 – 14 letters were observed from approximately week 8 and sustained to week 26 for all patients studied [119,120]

Despite the numerous trials conducted by Clearside Biomedical, at the time of writing only one other microneedle platform has reached clinical trials stage. The Phase 1 trial conducted by University Hospitals Leuven examined the potential for microneedle-mediated delivery of ocriplasmin, targeting patients suffering from central retinal vein occlusion. Importantly, the administration of the microneedle was controlled by a robotic system, ensuring correct alignment of the needle, and a pump to control the rate of infusion. In all four patients studied, a greater than 20% reduction in macular edema was observed at 26 weeks, demonstrating microneedle-mediated delivery of ocriplasmin to be feasible. One case of microneedle tip breakage did however occur, indicating potential safety concerns regarding the microneedle itself, as opposed to the formulation aspects [121]

From the numerous studies outlined above, and clinical success already being achieved for suprachoroidal space delivery, it is clear microneedles do show promise for ocular drug delivery. But why has clinical success not been more widespread? The remaining sections aim to address this question and offer perspective on areas that could aid such success. Particular focus has been made on the sclera as an application site, as it has received significant attention.

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6. Challenges in the clinical translation of ocular microneedles

Despite the promise of microneedles for ocular drug delivery, several issues still need to be addressed to enable successful translation of these drug delivery systems from the research laboratory to a clinical setting.

First, we believe we must better understand the biomechanical properties of the ocular tissues into which we are applying these microneedles and particularly how these changes depend on

location (e.g., at the limbus versus the equator of the sclera). Such properties will affect both the force of needle insertion required and depth of penetration achieved, as well as recovery rates. These factors must be fully evaluated to produce optimally designed microneedles for effective drug delivery and achieve better consistency of microneedle application and drug delivery. For example, since the sclera is highly elastic in nature, microneedle design parameters (e.g., needle height, needle geometry, interspacing, etc.) must therefore be carefully considered since these parameters will affect how the needle penetrates through the sclera.

Furthermore, the biomechanical properties of ocular tissues are also reported to change with age and under various disease states [122]; thus, this must also be taken into consideration when developing an optimal microneedle design since one specific set of microneedle design parameters will not be optimal for all ocular microneedle applications.

Furthermore, to improve the consistency of application, optimization of microneedle administration must also be considered. Patient acceptability and comfort must be central to the development of these application devices, particularly where the end goal is self-administration. This can be achieved through the development of applicator devices that can be designed to deliver at a consistent force or speed of injection. It is also desirable to minimize the number of steps involved in the administration procedure to improve patient acceptability and avoid the potential for injury or incorrect insertion, such as the patient applying excess force, which leads to pain and has further repercussions in terms of reduced acceptability and compliance.

As discussed, there are also challenges specific to each microneedle type that need to be considered. For example, hollow microneedles, due to clogging of the needle tip with tissue, require critical retraction distances to be established to enable infusion of the drug formulation [58], which is difficult to achieve clinically.

The loading capacity of the microneedles must also be considered to ensure that therapeutic levels are achieved and then sustained for the desired duration. By nature, microneedle arrays are small drug delivery platforms and therefore drug loading is limited by the surface area of these arrays, i.e., the number of needles. This is particularly true in the case of solid microneedles, which are restricted in terms of the doses they can deliver owing to the limited surface area of the arrays. Alternatively, hollow microneedles are not limited by the volume of formulation that can be delivered through their central bore; however, once in the sclera, the success of delivery depends on the ability of the drug to overcome the barriers of the eye.

Achieving accurate dosing is also a challenge, particularly for the delivery of biologics, which are more prone to degradation and stability issues than small molecules [123]. Such issues warrant careful consideration of microneedle fabrication procedures, microneedle formulation (i.e., the inclusion of stabilizers), storage conditions and packaging and transport conditions.

In summary, to enable more successful translation of ocular microneedles for clinical application, the microneedles design optimization vs biomechanics of ocular tissues must be thoroughly understood. Such optimization processes can be aided by innovative technology, such as finite element analysis, thus requiring a new outlook on how we can develop microneedle systems versus traditional approaches. The following sections will focus on these aspects, providing perspective on how these can act to improve the likelihood of preclinical and clinical success. [12][13][14][15][13][16][23][23][24][25][32][124][125][44][45][46][46][47][48][44][48][17]

[19][18][49][51][52][52][53][54][55][56][57][57][58][59][60][61][62][63][64][65][55][58][51][66][25][67][68][69][69][70][71][57][57][72][73][67]

7. Design considerations for the optimization of ocular microneedles

Irrespective of the type of microneedle in question, it is paramount that the microneedles possess suitable mechanical properties (i.e., toughness) to penetrate the target tissue without inducing partial or complete breakage of the microneedles. Not only is this crucial to avoid potential safety concerns, such as microneedle fragments remaining in the tissue but also in aiding patient acceptability of microneedles, particularly where self-administration is the desired target.

Despite the intended application, the overarching aim of microneedle administration is to minimize the invasiveness of drug delivery and achieve localization. To do so, the design of these microneedles must be optimized to lower the insertion force required to overcome the target tissue, which involves the need to overcome several forces, such as frictional forces. In addition to a detailed understanding of tissue biomechanics, optimization of the microneedles themselves plays a key role in fulfilling this aim. There are several microneedle design parameters that should be considered, including materials, needle geometry and bevel/tip angle, which will be discussed in more detail within this section. Figure 11 shows the relationship of these parameters in relation to therapeutic efficacy.
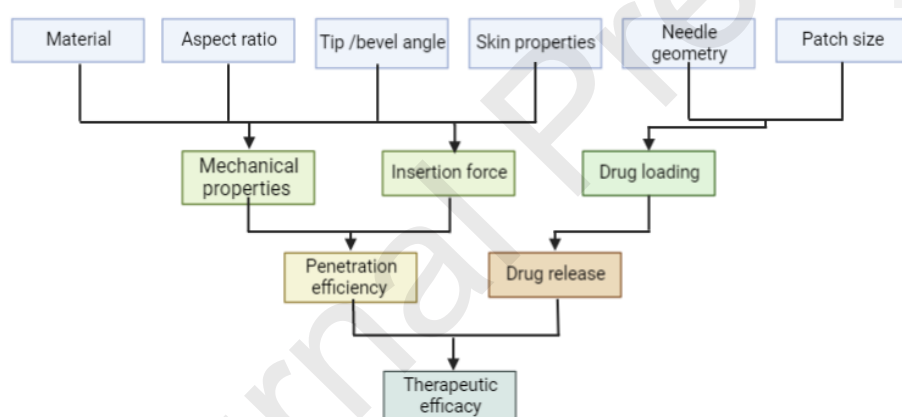
Figure 11. Schematic representation of design parameters to consider for micro to therapeutic efficacy, adapted from Johnston et al. [126].

The investigation of optimization of microneedles for ocular applications has been limited thus far in the literature; therefore, this section will look at how optimization has been completed for transdermal microneedles and how similar approaches should be applied for ocular microneedle design optimization.

First, the material selected for the fabrication of the microneedles will be a key determinant in the resulting mechanical properties. For example, hollow microneedles, as with traditional hypodermic needles, are commonly fabricated from stainless steel type 304 [127], which has a reported Young's modulus of approximately 190 GPa [128]. These values are above those

reported for both the skin (~ 0.33 – 1.28) [125][129]) and sclera (~ 2.5 – 6 MPa) [126][130]), which indicates that bending or fracture of the needle upon insertion, under safe and clinical predefined forces, should not occur. In agreement with this, studies have shown hollow microneedles fabricated from stainless steel to withstand axial forces in the region of 5 N [131]. Similarly, for dissolving microneedles, Albadr et al., demonstrated the ability of PVA/PVA/hyaluronic acid 3 x 3 microneedle arrays with 750 µm and 300 µm interspacing to withstand a force of 4 N without fracturing or bending. Mechanical properties were also shown to be improved by including PVA in the formulation [132].

Needle geometry has also been shown to influence the overall mechanical properties of microneedles. In the literature, several needle geometries have been investigated, including pyramidal and conical needles, as well as combined geometries such as needles with a cuboidal base and pyramidal tips, as shown in Figure 12a [112]. Li et al., investigated the influence of needle geometry on the mechanical properties of dextran dissolving microneedles used for the delivery of OVA for transcutaneous immunization purposes. They found that conical dextran-based dissolving microneedles provided enhanced insertion efficiency compared to other geometries studied (cylindrical base with cone tips, hexagonal base with pyramid tips and rectangular base with pyramidal tips) and stimulated greater immunoglobulin G production. This can be largely owed to the larger volume ratio provided by the conical needle structures compared to the other designs, meaning higher drug loading could be achieved [134]. More recently, De Martino et al., studied the effect of geometry on the insertion of PEGDA microneedles into porcine skin *ex vivo*. They found a 'star' base design to show improved penetration ability, regarding percentage insertion of the needle and compressive forces required to cut through the tissue, versus other shapes studied, such as 'circular' or 'triangular' bases, which was due to a positive correlation observed between the sum of the vertices and penetration capabilities [135].

Meanwhile for ocular applications, Amer et al., developed PVA-based hydrogel microneedles that incorporated an 'interlocking' region that, upon swelling (Figure 12b), secured the needle in place to enable sustained delivery into the vitreous cavity [136]. Furthermore, several bioinspired needles have been developed for transdermal applications, including a design inspired by a honeybee stinger that demonstrated enhanced penetration of the skin (Figure 12c), which should be considered for ocular administration [137].
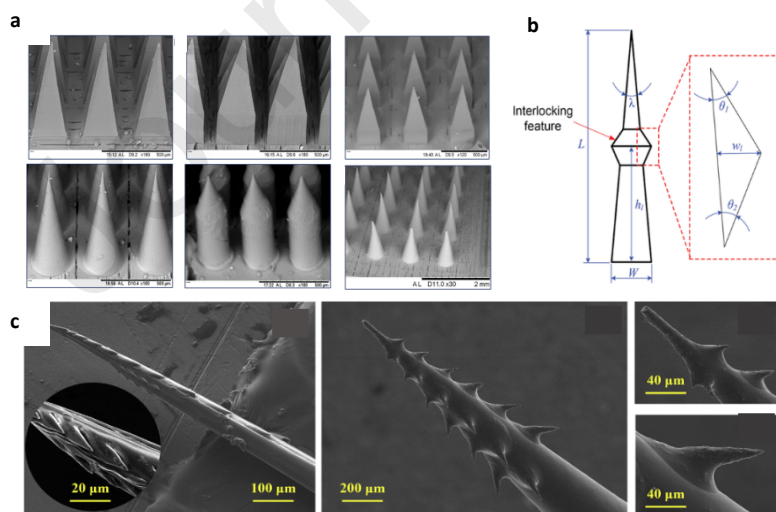

32

Figure 12. a) SEM images of polymeric microneedles of varying geometries [76], (b) Design of dissolving microneedle with interlocking feature to aid intravitreal administration [138]and (c) SEM images of bioinspired microneedle [80].

In addition to the geometry of the needles themselves, the sharpness of the needles will contribute to the efficiency of insertion. The sharpness of the needle can be defined by the bevel angle and by the tip angle for hollow and dissolving microneedles, respectively. In the case of hollow microneedles, Lee et al. used stereolithography combined with laser cutting to engineer stainless steel hollow microneedles with precise bevels (0, 30 and 60°, measured from the datum) and an ultrahigh aspect ratio for blood sampling. They found the 60-degree bevel, the sharpest needle, to enable better penetration while also being able to withstand axial loads of approximately 5 N [131].

The length of the microneedles should also be optimized based on the target site for application. In the case of the cornea, sclera and suprachoroidal space, needles must be long enough to effectively penetrate these tissues without fully penetrating them. Biological variation makes this difficult to achieve clinically, since tissues such as the sclera are known to vary in terms of thickness between individuals. To address this issue within a clinical setting, Clearside Biomedical performed a study assessing the clinical acceptability of performing suprachoroidal space injections using XIPERE®, a single metallic hollow microneedle, which was recently approved in 2021 [139,140], and their customized SCS Microinjector® device. Following the administrative protocol, patients were first injected with a 30G microneedle measuring 900 μm in length perpendicular to the scleral surface, with successful insertion into the suprachoroidal space characterized by a reduction in resistance during the insertion procedure. Out of all injections performed, 71% of injections were considered to have achieved successful penetration into the suprachoroidal space using the 900 μm long needle. All remaining injections were successfully completed upon switching to a longer needle length of 1100 μm when repeating the injection procedure [141].

In addition to the design characteristics of individual needles, the overall patch size will influence the mechanical behavior in the case of microneedle arrays. The number of needles and their subsequent interspacing can lead to a 'bed of nails' effect if the interspacing distance is too small, generally under 150 μm [142], which leads to inefficient needle insertion. Moreover, the patch size will also dictate the efficiency of needle insertion. Unlike skin, which has an average surface area of approximately 2 m^2 [143], ocular tissues have a very small surface area in comparison (less than 200 mm^2) [144]. Additionally, unlike the skin, which provides a comparatively flat site for microneedle array application, administration of the arrays to the eye is further complicated by the surface curvature of this organ, thus limiting the patch size that is capable of effective needle penetration. To the best of our knowledge at the time of writing, the maximum patch size for the eye and the effect of curvature on microneedle penetration efficiency have not been investigated. Furthermore, unlike the skin, which has a solid support for microneedle insertion, the eye acts as a hydrostatic cavity that will compress under applied forces. The force required for microneedle insertion must be safe to prevent excessive changes in intraocular pressure; however, an acceptable and safe value has not yet been defined. Furthermore, it is yet to be established whether a transient increase in intraocular pressure above physiological levels is safe and acceptable and the long-term effects of this.

In the case of dissolving microneedles, the shape and height will also affect the capacity for drug loading, since the drug should be loaded primarily within the needles themselves, opposed to the baseplate, to prevent drug wastage. To enhance drug delivery using dissolving microneedles, techniques such as centrifugation have been employed to concentrate the formulation at the tip of the needles, as was done with OVA-loaded PLGA particles in a PVA/PVP dissolving microneedle array developed by Wu et al. [19]. While the needle size can be increased to improve the drug loading, care should be taken since changing the needle size will ultimately change the aspect ratio, defined by the ratio of microneedle length to base diameter. This will ultimately affect insertion efficiency, with high aspect ratios demonstrating reduced insertion forces compared to those with lower ratios [145]. Therefore, optimization using the characterization techniques outlined earlier in the review should be considered. This reiterates the importance or benefit of delivering small molecules with high potency.

Although optimisation of microneedle design is crucial, it must be noted that due to changing biomechanical properties of ocular tissues, one 'optimised' needle cannot hold true for all applications. It is therefore also important to understand the influence of tissue biomechanics on microneedle administration and drug delivery. The following section aims to highlight such matters, using the sclera as our primary focus given the interest it has received as a site for microneedle administration in the eye. Research into sclera biomechanics is, however, in its infancy, with much still to be understood. We therefore also highlight what is already known about skin biomechanics, which has been much more greatly characterised, to highlight learnings that we can take form transdermal applications, similarities in comparison to the sclera, and gaps we need to address in ocular biomechanics research.

8. Learnings from transdermal microneedles

By looking at transdermal approaches for microneedle applications, which have been studied since the late 1900s, including comparisons between skin and ocular tissue biomechanics, we may begin to understand how to translate these platforms for ocular applications.

8.1 The skin as an application site for microneedle platforms

Since first being utilized in a clinical setting in the late 1970s [146], transdermal drug delivery has gained attention given the several advantages it possesses over oral drug delivery, such as avoiding first-pass metabolism and providing sustained drug delivery profiles [147]. However, the efficacious delivery of drugs across the skin is challenging [89], due to the strict physiochemical properties a drug must possess to be effectively absorbed across the skin [148].

The barrier function of the epidermis can be primarily due to the 'brick and mortar' structure contributed by the corneocytes and lipids within the stratum corneum layer. The stratum corneum is a thin (10 – 16 μm thick) [149] but essential protective layer of the skin. [92] From a drug delivery perspective, the brick-like, ordered arrangement of the corneocytes within the stratum corneum creates a highly tortuous route for the diffusion of drug through the transcellular route. [95]

Underneath the epidermal layer lies the dermis, which is predominantly composed of fibrous components, such as collagen and [93] elastin. These components are essential in providing flexibility of the skin. The presence of collagen fibers, which account for approximately 75% of the dermis dry weight, impart strength to the skin [150] and are predominantly of type 1 and type 3 collagen [151]. Moreover, the thickness and density of collagen fibrils/fibers is also heterogeneous within the dermis, increasing from the thinner outermost papillary dermis to

deeper into the skin toward the reticular dermis [152]. Meanwhile, elastin fibers, present at much lower concentrations (~2% of skin dry weight) [153], are also responsible for establishing the flexibility of the skin tissue. Additionally, the dermis is populated with proteoglycans, making up only approximately 0.1 – 0.3% of the skin's weight [154], with decorin, biglycan and versican being the most abundant [155]. Proteoglycans play an essential role in maintaining the hydration of the skin and influence its responses to compressional forces by generating swelling pressure. Therefore, in addition to the flexibility of the skin, its biomechanical properties are largely determined by the dermal layer.

Since first being used for drug delivery, the development of microneedle arrays has been focused on transdermal applications. The short length of microneedles means they only penetrate the outermost epidermal layers of the skin, thus avoiding puncturing the dermal layer, the disruption of which would lead to pain upon insertion. To enable successful translation of transdermal microneedle systems into clinical translation, understanding of the tissue biomechanics is essential, since such properties will affect needle insertion forces and tissue recovery.

8.2 Biomechanical properties of the skin

Understanding the biomechanical properties of soft tissues, such as the skin, is an essential step in providing insight into how the tissue will behave in response to various mechanical loads. In relation to microneedle development specifically, the biomechanical properties of the tissue will play a role in determining the insertion forces required for microneedle arrays and how the tissue will recover following insertion (e.g., pore closure times). For a detailed review of characterization methods for tissue biomechanics, we refer you to a review conducted by Boote et al. [156], which outlines characterization methods for sclera biomechanics but is equally applicable to other soft tissues, such as the skin.

Due to the presence and arrangement of collagen and elastin fibers within the dermis, the skin can be described as an incompressible, hyperelastic material that displays anisotropic properties [157]. Given the anisotropic nature of the skin, microneedle development can be optimized in terms of microneedle design and application site, for example, to achieve better insertion and consistency of delivery.

The collagen fibers in the dermis are tightly packed, however, which can create difficulties regarding their characterization, especially in terms of fiber orientation. As a result, and due to variations in testing methodologies, the quantitative orientation of collagen fibers in the dermis was largely disputed. Recently, in an attempt to overcome this issue, Ueda et al., used a combined characterization approach of biaxial testing methods with multiphoton microscopy to characterize fiber orientation in human dermis. Two to three modes of collagen fiber distribution were observed within the superficial layer of the dermis, with a major distribution orientation of fibers that was intersected by a much smaller number of fibers. A similar pattern was observed for elastin, whereby the elastin fibers showed a close alignment with the collagen fibers in the superficial dermis (94% ± 4%), which decreased in the deeper reticular dermis (85% ± 10%) [158].

As with reports of fiber orientation, the elasticity of the skin, characterized by the Young's modulus, has been reported over a wide range due to differences in methodologies employed. Methods that employ torsion tests have produced Young's modulus values between approximately 0.4 and 0.8 MPa, uniaxial test methods have reported much higher values in the range of MPa (4 - 20 MPa) [159]. Additionally, the location of the excised skin sample used

for testing may also contribute to variation in reported values since fiber orientation is known to be location dependent [160]. Furthermore, given the anisotropic nature of the skin, its failure will occur in a manner that is dependent on the direction of the applied load and the rate of strain. For example, the failure rate of skin samples excised from the human back was 0.06/s at a low strain rate versus 167/s at higher strain rates [161].

Therefore, such biomechanical properties are important to understand and optimize microneedle insertion into the skin. For example, it has long been established that the hydration level of the skin also affects Young's modulus, with increasing hydration leading to a decrease in modulus [162]. It is also well established that both increasing age [163] and disease states (e.g., diabetes) lead to changes in biomechanics, such as reduced elasticity [164]; thus, these changes must also be understood to aid in the effective development of microneedle systems.

Importantly, lessons can be learned from transdermal microneedle development and characterisation of skin biomechanics, of which there is more developed knowledge, due to the similarities between skin and sclera tissue. Similarities and differences of the sclera to the skin will be highlighted in the next sections, which can be used to inform the translation of microneedles towards ocular applications.

9. Translating transdermal microneedles to ocular applications

As outlined thus far, microneedles have been used to deliver a wide variety of formulation types, including solutions [165], nano/microparticle formulations [165], gene therapies [166] and thermoresponsive polymers [167] to the eye. Moreover, these platforms have been utilized to target localized delivery of drugs to several ocular tissues within the eye, including the cornea and sclera, as well as the suprachoroidal space, to provide sustained delivery of therapeutics. For example, the first microneedle for the eye (XIPERE™, Clearside Biomedical) was recently approved in October 2021 [168] for administration of triamcinolone acetonide to the suprachoroidal space. Despite this, there are still clinical concerns regarding the potential for ocular hemorrhage, infection and inflammation associated with administration into the suprachoroidal space using microneedles [169]. Therefore, load-bearing tissues such as the sclera could act as a potential site for localized delivery of therapeutics to provide sustained release to the retina.

The use of microneedle platforms for ocular drug delivery; however, is not without challenges, and as described previously, their translation to clinical settings has been limited. For example, for the successful instillation of a drug into the sclera using hollow microneedles, a critical retraction distance must be established [170], which is difficult to achieve clinically. To enable more successful clinical translation of ocular microneedles, a greater understanding of challenges, particularly those posed by tissue biomechanics, is needed.

9.1 The sclera as an application site for microneedle platforms

The sclera is a fibrous, avascular tissue with a surface area of approximately 17 cm^2 [171]. Despite having a much smaller surface area than that of the skin, the sclera constitutes one of the largest static barriers to posterior segment drug delivery.

As with the skin, the sclera plays a key protective role but also has organ-specific roles, such as maintaining the globular shape of the eye, in coordination with outward pressure from the vitreous humor, which is essential for the normal focusing of light toward retinal photoreceptor cells [172].

The sclera is also multilayered in nature and comprises the episclera, stroma and lamina fusca (Figure 13a and b). Additionally, in comparison to the skin, the sclera lacks the brick-and-mortar structure observed in the skin. Due to this, the scope of drug molecules that can successfully penetrate through the sclera is larger than those for the skin. Figure 13c and d show the difference in structure between the skin and sclera taken from histological sections.

The episcleral layer constitutes the outermost layer of the sclera, which comprises a loose, fibrous network of both collagen and elastin fibers. This layer also contains vasculature in the form of episcleral veins, which have been shown to play a role in the elimination of drugs administered *via* periocular routes [173]. Beneath the episclera resides the stroma, the largest layer of the sclera, which also contains an extensive network of collagen fibers but lacks the vasculature observed in the episcleral layer. The stroma comprises collagen bundles that contribute to the overall nonlinear viscoelastic properties of the sclera, with type I collagen being the most predominant (95%) and other collagen types, such as type III, V and VI, being present at lower quantities (~ 5%) [156]. Collagen fibers, ranging from 25 to 230 nm in length, are arranged to create micron-thick (0.5 – 6 µm) lamellae that exist within a proteoglycan matrix and account for approximately half the weight of the sclera [156]. Unlike in the cornea, the collagen fibers within the stroma are arranged in a highly randomized manner, thus contributing to the opacity of the sclera and preventing the off-axial transmission of light. Elastin fibers also exist within this matrix at much lower concentrations yet impart a high degree of elasticity to the sclera. The highly elastic nature of the sclera has been quantified by techniques such as atomic force microscopy, which is necessary to enable the sclera tissue to expand in response to any increases in intraocular pressure [174].

Similar to the skin, another major vital component of the scleral stroma is proteoglycans, with biglycan, aggrecan and decorin being the most abundant [175]. Given that the stroma accounts for the largest region of the sclera, it is considered to be the most important to consider regarding transscleral drug delivery targeting posterior segment tissues. Finally, the lamina fusca, the innermost layer of the sclera, is a thin pigmented tissue that provides a supply of oxygen and nutrients to the retinal layer through its physical connection to the choroid.

As with the skin, the thickness and composition of the sclera is location dependent (Figure 14). Scleral thickness is greatest at the posterior pole (1–1.35 mm), while the thicknesses at the limbus and equator are approximately 0.8 mm and 0.4 – 0.6 mm, respectively [176]. The scleral thickness also decreases at the insertion points of the rectus muscles (0.3 mm) and tendon fibers (0.6 mm) [176]. The thickness of the sclera has been shown to change with age and is also implicated in certain disease states, such as glaucoma [177].
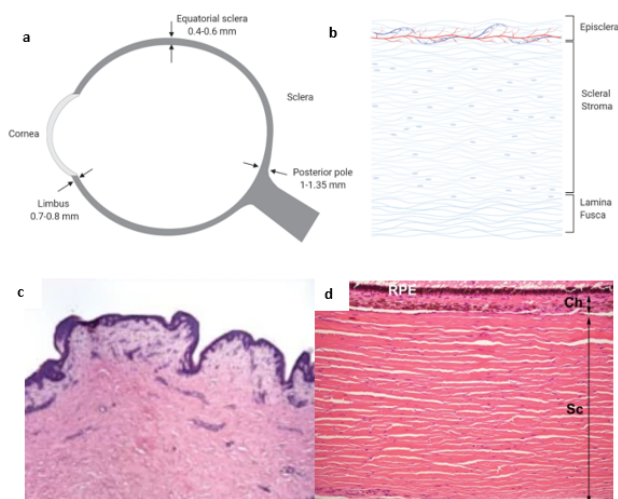
Figure 13. (a) Schematic representation of location-dependent changes in average sclera thickness values across a human eye, (b) Schematic representation of the sclera structure, showing the episclera, stroma and lamina fusca layers comprised of a collagen network embedded in an aqueous matrix, and histological images of the (c) skin and (d) sclera [178]

Numerous physiochemical properties, particularly the charge and molecular radius of drug molecules, are important factors in determining transscleral permeation [179]. For example, it has been demonstrated that scleral permeability shows inverse proportionality to the molecular radii of drug molecules [180], with an upper limit of approximately 70 kDa [181]. This is supported by work conducted by Geroski et al., which showed small molecules (fluorouracil, 5-FU, Mw: 130 Da) to readily permeate through the aqueous matrix of the sclera $(43.9 \times 10^{-6}$ cm/s), while larger proteins (dextran, Mw: 70,000 Da) show decreased diffusivity $(1.9 \pm 10^{-6}$ cm/s)[60]. Furthermore, the presence of negatively charged proteoglycans under the natural physiological pH of the sclera poses another challenge for transscleral drug delivery, particularly for drugs or delivery systems, such as nanoparticles, which bear a positive charge [62]. Many biologics administered for the treatment of posterior eye diseases, e.g., bevacizumab (Avastin, Genentech Inc., USA), are positively charged; hence, they are prone to forming polar interactions with proteoglycan molecules and demonstrate reduced transscleral permeation [62].

As a load-bearing tissue, the sclera also provides structural support to more delicate, underlying tissues, such as the retina, which possesses a lower Young's modulus than the sclera (1.3 – 26 kPa vs 1.8 – 2.9 MPa) [63][64]. To carry out such roles, the biomechanical properties of the sclera (e.g., elasticity and tensile strength) and their maintenance are essential. There is a current need to better understand scleral biomechanical properties, which would enable the development and clinical translation of microneedle platforms targeted to the eye.

Despite the promise of this tissue site for application, the type of microneedle for scleral administration must be carefully selected. While hydrogel-forming microneedles have been used extensively for transdermal applications [65], they are likely to be unsuitable for ocular applications. Given the highly hydrated nature of the sclera and the constant presence of a hydrated layer on top of the scleral surface, the microneedle could potentially swell before

38

complete insertion, leading to reduced sharpness of the tip and a negative impact on mechanical properties, which leads to incomplete and inconsistent insertion.

9.2 Biomechanical properties of the sclera

As with the skin, the biomechanical properties of the sclera can be largely owed to the arrangement of collagen and elastic fibers within this tissue. Therefore, the sclera is also characterized as an incompressible, hyperelastic material with anisotropic properties, and as with transdermal microneedles, there exists the opportunity to identify the most suitable application site of ocular microneedles to the sclera to improve needle insertion and consistency of delivery. Such rationale would be influenced by the mechanical properties of the sclera at different regions, and by relevancy to clinical application. For example, the site must be easily accessible for microneedle administration, limiting application of these platforms towards the front of the eye.

Over recent years, there has been a growing interest in better characterizing the sclera tissue using both *ex vivo* and *in vivo* approaches; however, much remains unknown about this tissue, such as deformation behavior, and conflicting findings have occasionally been reported in the literature because of methodological variation (e.g., effect of sample preconditioning) [66]. Other factors, such as interspecies variation in biomechanical properties, should also be further investigated and considered, with previous literature findings indicating significant differences in the stiffness of mouse and porcine sclera under various strain rates, for example [67]. It is important to understand and consider factors such as interspecies differences for experimental testing on *ex vivo* models and their translation into preclinical testing.

Thus far, the majority of collagen fiber arrangement analyses within the sclera have been conducted close to the optic nerve to monitor biomechanical changes during glaucoma. Coudrillier et al., used wide-angle X-ray scattering to map the degree of collagen fiber alignment in the peripapillary sclera and found the degree of alignment to be heterogeneous in this region [68]. It is, however, still well established that collagen fiber organization is highly randomized across the entire sclera [69] and not just surrounding the posterior pole. Such arrangements are also location dependent; for example, toward the limbus, the collagen fibers are arranged in a circular orientation to aid corneal curvature [70].

Techniques such as polarized light microscopy have shown the highly interwoven nature of these collagen fibers, which helps to impart strength to the sclera tissue [71]. As with the skin, the arrangement of collagen, and thus the biomechanical properties, of the sclera have been reported to be influenced by several factors, including disease state (e.g., glaucoma and myopia) and level of tissue hydration [32]. Furthermore, as a result of aging, other parameters, such as the degree of collagen crimping, the morphology of which is an important determinant of biomechanical responses, also decrease with age, which leads to reduced elasticity of the sclera [72].

Through uniaxial testing methods, an increase in stress has also been observed in scleral samples excised from older human eyes. Such increases are also location dependent, with anterior segment samples demonstrating the greatest increase in stress in eyes over 50 years versus samples taken from younger eyes [73].

Collagen fiber thickness has also been shown to change depending on the location within the sclera. Park et al., conducted second harmonic generation imaging of the sclera at differing depths (5, 35, 75 and 90%), moving from the conjunctiva to the superficial sclera, finding collagen fiber thickness to increase significantly between 5 and 90% of the sclera depth [194] This could indicate that the sclera becomes more difficult to penetrate as it reaches the lower layers of the sclera.

Even in the absence of aging or disease state, biomechanical properties of the sclera have been shown to vary within different quadrants of the same eye. After taking repeated measurements along the equator of the eye, differences in the elastic modulus of the inferior quadrant versus the temporal quadrant were established, with values of 1.84 ± 0.30 and 6.04 ± 2.11 MPa, respectively. Differences in deformation behavior between the quadrants were also reported, with deformation occurring most rapidly within the inferior quadrants [74]. This is expected given that more elastic material (i.e., lower elastic modulus) will deform more rapidly. As expected, deformation in the posterior region of the sclera was lower than that in other regions tested [74], which may be due to the increased thickness of the tissue in this region. These findings therefore support the idea that it is possible to optimize the location of microneedle administration to a region of the sclera where tissue recovery will be faster (more elastic) while still possessing sufficient rigidity to aid needle insertion at a safe force.

However, while the biomechanical properties of the sclera have been well reported in the literature regarding changes undergone with aging and in disease states, little research has been conducted regarding biomechanical influences on microneedle insertion. Understanding interactions between the microneedles and the microstructural components of the sclera could aid in the development of optimized microneedle platforms for ocular drug delivery, both in terms of microneedle design and drug delivery. In addition to microneedle design optimization, there is also a need to better understand the tissue behavior of the sclera, particularly its deformation behavior under various loads.

Techniques such as computational modeling can therefore be employed to aid in the optimization of ocular microneedle design and in understanding how the sclera behaves in response to loads applied by the microneedles. While computational modelling approaches have been developed for transdermal microneedles, the approach has not yet been widely used for the development of ocular microneedles. This is primarily due to the lack of *in vivo* data available for tissues such as the sclera which is required for model validation purposes. This again highlights the importance of learnings in comparing skin and sclera structure and biomechanics, since, due to the similarity of the two tissues regarding collagen fibre contribution. It is feasible to expect that knowledge on the skin may be used to inform such models in the early stages of development where similarities in biomechanics can be established.

10. Computational modeling for optimization of microneedle design, application and drug delivery aspects

Computational modeling is a combination approach, utilising computer science, physics and mathematics, to simulate real world systems [196]]. One of the most popular types of computational modeling is finite element analysis, or finite element modeling. Finite element

modelling can be described as an approximation method that divides structures of interest into small elements, of which the behavior of these elements can be explained mathematically using partial differential equations.

In the process of developing a finite element model, researchers first develop a 3D CAD model of the object. The object is then divided into a given number of units, or 'finite elements', which are interconnected and joined by 'nodes'. Material properties are then applied, as well as relevant boundary conditions that define the physical constraints of the model. During the simulation, the software solves equations explaining the behavior of the individual elements, which are then summated to produce a general solution for the whole model. Such solutions can then be analysed during postprocessing, through quantitative data or contour plots, for example [75]. To ensure accurate and reliable results, each model must also be validated by demonstrating the correlation between the simulated and experimental results.

Historically, finite element modelling has been widely used within automobile and aerospace industries since the 1950s to produce reliable estimations and simulations of how materials used in the design and fabrication of such structures will behave under stress [76]. Since then, the potential for finite element analysis to aid in the development of drug delivery systems, as well as the design optimization of medical devices, has been widely recognized and investigated.

Finite element analysis offers several advantages within the field of drug delivery, specifically for microneedle development and optimization. Several clinical challenges currently exist when attempting the clinical translation of microneedle technologies, such as the time-consuming and costly nature of microfabrication techniques [77]. Finite element analysis software enables the ability to prescreen microneedle designs and material properties prior to fabrication, saving both time and resources. It also enables easy adaptability of microneedle design during testing, as well as simulation of drug release profiles prior to *in vivo* studies.

Within ocular drug delivery, finite element analysis has been used to optimize implantable ocular devices, such as implants, and optimize the design parameters of contact lenses. For example, Batalu et al., used finite element analysis to compare the safety profile of contact lenses that adopted various designs and polymeric materials under different application pressures [78]. This approach enabled a means to efficiently prescreen potential product designs prior to any fabrication and experimental testing. Overall, this reduces the number of experiments that need to be conducted, which may help to reduce the costs and time associated with product development. However, the accuracy of such models is limited by the quality of experimental data used to validate the model, which emphasizes the importance of more realistic *ex vivo* models in the development of microneedle systems.

In terms of using computational modeling in microneedle development, the aim is to provide quantitative analysis of how the microneedle interacts with the target tissue. This can allow us to understand tissue deformation and stress distribution aspects under different conditions. For example, such models enable the ability to simulate tissue deformation, which will be dependent on tissue biomechanics.

To the best of our knowledge, only one paper has used finite element model to aid in the development of ocular microneedles. Park et al., used modelling to confirm experimental findings by predicting the diffusion patterns in the sclera following the application of rhodamine B-coated solid microneedles. Through simulations, they found that insertion depth influenced rhodamine B distribution, whereby both the delivered volume and distribution of

rhodamine B were greater at deeper penetration depths (Figure 14a). Importantly, such findings were conclusive with experimental data, validating the simulated model [50].

Even within the field of transdermal microneedles, despite the promise of finite element modelling as a tool in assisting the development of optimized microneedle platforms, only 7% of papers published on microneedles between 2000 and 2020 were related to modeling applications. This is compared to 26% focused on fabrication methods and 58% on experimental papers [3]. Therefore, there remains great potential to incorporate finite element modelling into microneedle development for optimization purposes, particularly for ocular applications.

Within transdermal microneedle development, finite element modelling has been used to analyze the stress distribution of the skin following microneedle application, which has been shown to be dependent on microneedle design parameters (e.g., needle geometry) and hence can be used to optimize needle design. Such studies should be considered and translated to ocular microneedle applications. For example, Loizidou et al. studied how changing the needle geometry affected the mechanical properties of the needles. They found that designs with an increased number of vertices (i.e., hexagonal and polygonal) displayed more robust mechanical properties than those with fewer vertices (i.e., triangular) [79]. In addition to conventional microneedle geometry, more novel and bioinspired designs have also been investigated for microneedle design strategies, which have been aided by finite element modelling. Chen et al., used modelling to assess the stress distribution of the skin immediately surrounding the insertion site of various bioinspired 'barbed' microneedles. Simulations involving the bioinspired needles were found to minimize the frictional force, thus reducing the insertion force of the needles, as well as improving retention in comparison to the 'barbless' control needles (Figure 14b), thus enabling the prescreening of such designs prior to fabrication [80].
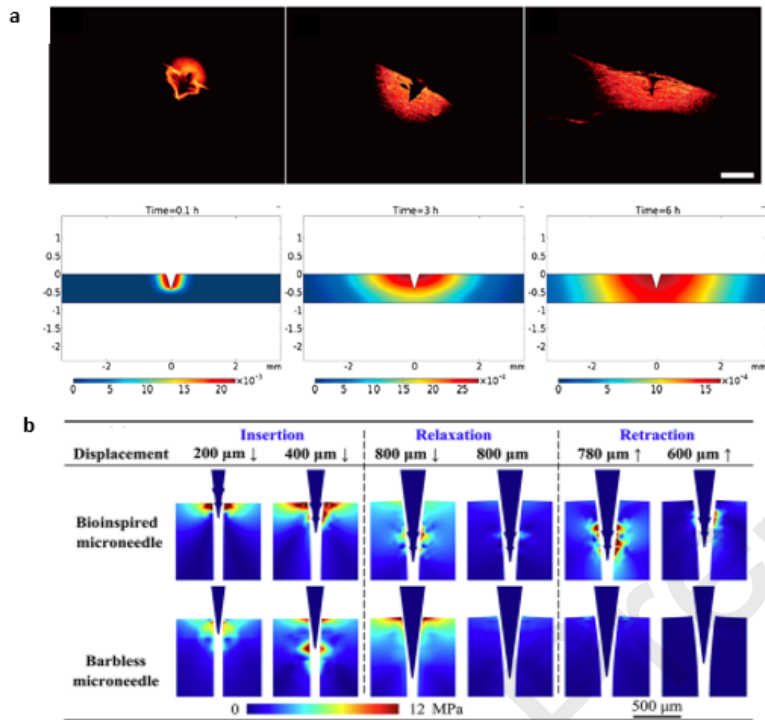
Figure 14. (a) Time-dependent experimental distribution profile of RB fluorescently stained scleral tissue samples versus simulated distribution profile following rhodamine B administration using the microneedle system with a 'medium' spring constant [50], and (b) stress distribution profile of bioinspired barbed and control microneedles during insertion and retraction processes [80].

As discussed previously, it is important to understand the mechanical capabilities of the microneedles so as to avoid breakage. Such properties are typically characterized experimentally using texture analysis; however, finite element modelling has also been used to study the mechanical behavior of microneedles. In further work conducted by Loizidou et al., the von Mises stress and buckling forces were analyzed in microneedles fabricated from 1:1 ratios of carboxymethyl cellulose (CMC) in combination with different sugars (sucrose, maltose, and trehalose dihydrate). As expected, a trend between the critical flexion load of the microneedle and the Young's modulus of the incorporated sugar was discovered, with maltose-based needles providing the best mechanical properties [201]

Such studies provide an example of how finite element modelling has been used to optimize the design of transdermal microneedles and thus highlight the potential and importance of conducting similar analyses for the future development and optimization of ocular microneedles.

11. Regulatory aspects

Much of this review has focused on issues limiting ocular microneedle development and translation on a laboratory scale. Despite the promise an ocular microneedle platform may show there may be one last problem limiting its clinical potential: lack of GMP standards. This raises the question of how we define microneedles, and thus how we then regulate such systems. Namely, there is a lack of clarification on whether a microneedle is a drug product itself or simply a device used to deliver the drug. What makes this particularly challenging is the fact this may be interchangeable on a case-to-case basis. For example, hollow microneedle may be considered as a device through which the formulation is delivered; however, in the case of dissolving microneedles for example, polymeric components from which the microneedle is formed dissolve to facilitate drug release. Furthermore, the geometrical parameters that define what a microneedle is are somewhat blurred. In literature, microneedles have typically been described as being micron-scale needles of 1 mm or less; however, papers have explored the use of 'tower microneedles' which are much longer (up to 5 mm) [203–205]

Additionally, some issues regarding regulatory aspects concern the lack of standardisation for microneedle characterisation. Techniques discussed previously in Section 3 may vary in terms of instrument capability or even methodology, making it difficult to directly compare results.

These issues have been discussed in depth in our previous review p aper from the Donnelly lab, to which we refer readers for further detail [206].

Conclusion

The delivery of small molecules and biologics to the eye presents a complex challenge due to the presence of multiple ocular barriers and conventional formulations thereby limiting therapeutic efficacy modalities. Intraocular injections are commonly employed to overcome the barrier properties of the eye to enhance ocular bioavailability of therapeutics. In chronic conditions the need for frequent intraocular injections or eyedrops remains one of the biggest unmet needs in ophthalmology. Injections with conventional hypodermic needles are prone to significant challenges such as frequent administration, invasiveness, and reduced bioavailability as discussed in this review. To overcome these limitations microneedles, provide an alternative and effective approaches in treating a range of anterior and posterior segment eye diseases. Notwithstanding certain limitations the design and application of microneedles could offer several advantages over conventional drug delivery approaches. This is evident by raising interest in this field with ability for translate the technology to clinical application, as successfully demonstrated by the first product in the market (Xipere Microinjector®). However, further research is necessary to thoroughly understand the challenges faced by the eye on the design of microneedles, tissue properties in both healthy and disease eye, type of therapeutic to be delivered and extent of sustained release or localized delivery desired. Advanced tools such as FEM can play a crucial role in generating significant body of data may help the researchers in optimizing microneedle design appropriate for a given clinical condition. Overall, microneedles can change the way drug can be delivered to the eye and provided better therapeutic outcomes including improved patent acceptance. The administration of microneedles has been observed to not only impede the advancement of the disease but also mitigate the degeneration of the retina. Several recent pre-clinical and *in vitro* studies have demonstrated the utilisation of microneedles as drug delivery tool in the treatment of various ocular diseases.

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