A review of corneal biomechanics: Mechanisms for measurement and the implications for refractive surgery

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Detailed clinical assessment of corneal biomechanics has the potential to revolutionize the ophthalmic industry through enabling quicker and more proficient diagnosis of corneal disease, safer and more effective surgical treatments, and the provision of customized and optimized care. Despite these wide-ranging benefits, and an outstanding clinical need, the provision of technology capable of the assessment of corneal biomechanics in the clinic is still in its infancy. While laboratory-based technologies have progressed significantly over the past decade, there remain significant gaps in our knowledge regarding corneal biomechanics and how they relate to shape and function, and how they change in disease and after surgical intervention. Here, we discuss the importance, relevance, and challenges associated with the assessment of corneal biomechanics and review the techniques currently available and underdevelopment in both the laboratory and the clinic.

Key words: Biomechanics, corneal crosslinking, customized treatment, diagnostic screening, keratoconus, refractive surgery

The biomechanics of the cornea govern its shape and therefore its refractive power. Abnormalities and changes to biomechanics that present due to disease or are introduced due to trauma or surgery, can have profound effects on vision. Gaining a comprehensive understanding of how the mechanical properties of the cornea contribute to its shape has become increasingly important since the introduction of refractive surgery in the 1990s and its subsequent popularity, with over 60 million procedures undertaken worldwide to-date. Presently, the demand for technologies capable of clinical assessment of biomechanics has never been higher, due to the recent availability of treatments, such as corneal-crosslinking (CXL), capable of minimally invasive, direct topographic manipulation of corneal stiffness. With access to detailed, spatially specific information regarding patient's corneal biomechanics, and algorithms detailing the biomechanical implications of such treatments, they have the potential to be used for refractive manipulation, not only in the instance of refractive surgery and the treatment of keratoconus but also in cataract surgery and with cornel grafts, giving a potential market for this type of patient-customized treatment of 10's of millions of patients per year.

Biomechanics in the Diagnosis and Treatment of Disease

Keratoconus, a disease-associated abnormal corneal biomechanics, is estimated to affect between 1 in 400 and 1 in 2000 people.[1] In keratoconus, defects exist in the collagen structure and the surrounding matrix which result in a loss of biomechanical integrity, leading to progressive thinning and focal changes in corneal curvature. Currently, diagnosis of keratoconus is made by recognizing abnormalities and monitoring changes that occur to the topographic and pachymetric measures and to vision over a given time period [Fig. 1]. Treatment is then focussed on preventing further progression, with options including hard contact lenses, CXL, intracorneal ring segments, and in advanced cases corneal transplant.[2]

The availability of treatment options, such as CXL, which allow progression of the disease to be halted in the earliest stages, has sparked interest in technologies capable of early and specific diagnosis. Relying on the identification of changes to morphologic features, such as topography and thickness, for diagnosis is an inadequate approach requiring progression of the disease to the point where vision has deteriorated to some degree. Instead, focus needs to be directed at identifying the underlying biomechanical abnormalities that precede these changes. This is a major clinical challenge. Biomechanical abnormality in keratoconus is believed to be initially focal in nature[3,4] and it is this focal reduction in elastic modulus which begins a cycle of biomechanical decompensation. Hence, to identify and address the disease at its earliest stage, high spatial resolution screening methods are required, capable of examining spatial variations in biomechanics with high sensitivity.

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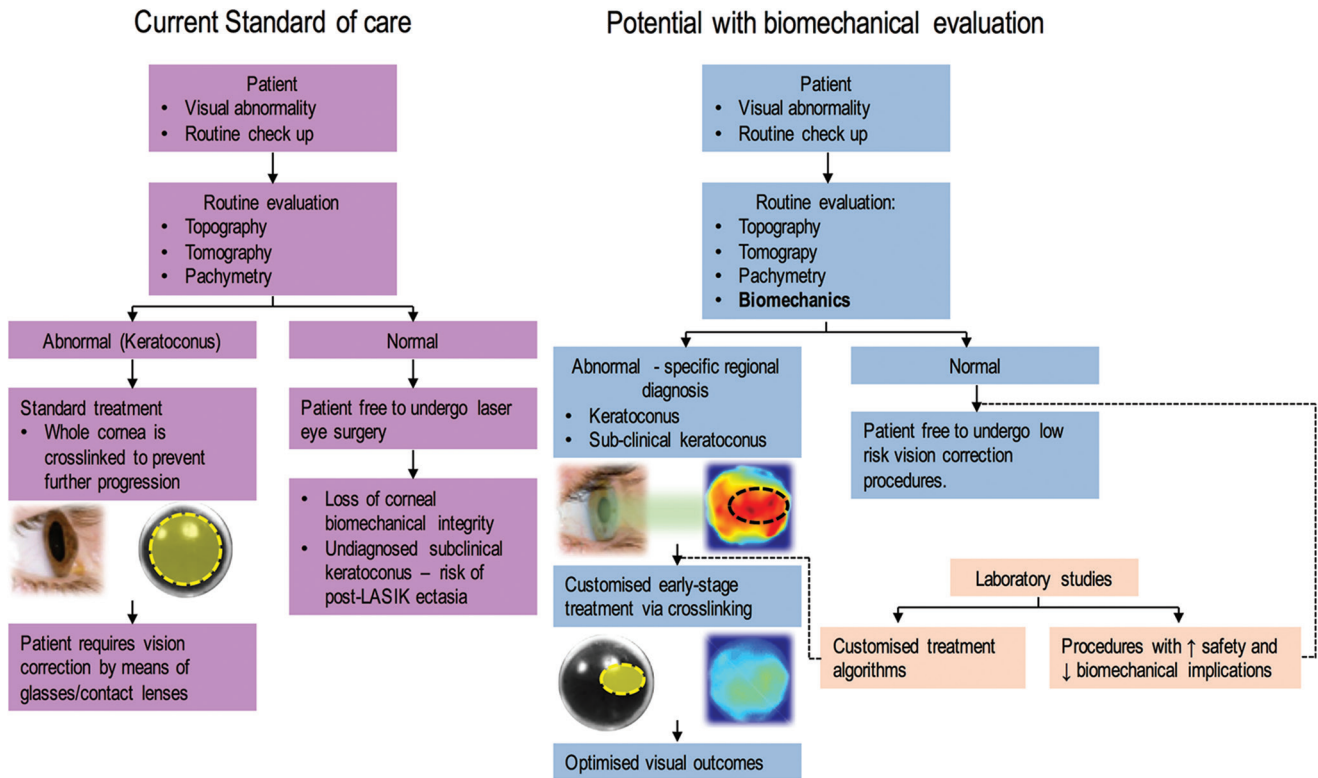
Figure 1: Potential changes to patient care with the availability of appropriate methods of biomechanical assessment in the laboratory and clinic

The specificity of diagnosis is important. If the exact location and severity of biomechanical weakness can be identified in an individual's cornea, then it opens the opportunity for customized treatments to deliver optimized visual outcomes [Fig. 1]. Treatments such as CXL have already demonstrated potential to deliver refractive modifications[5–7] and can easily be applied in isolated topographic locations.[8,9] To facilitate customization of treatments, a method is required to specifically diagnose abnormalities in terms of severity and location, along with a comprehensive understanding of biomechanics and the effects of these treatments. The latter of which is already being investigated in laboratory-based studies;[10] the aim of which is to provide the necessary treatment algorithms specifying variables such as power, treatment time, and treatment location to bring about accurate and predictable changes to refractive power.

These types of treatments if made available have the potential to not only prevent vision loss but also reduce the cost and burden of more invasive late-stage treatments, such as corneal transplant, associated with poorer visual outcomes.

Biomechanics in Corneal Surgery

Assessment and understanding of corneal biomechanics has application in corneal surgery for determining patient suitability and improving the safety and efficacy of current procedures.

In excess of 4 million people globally per year undergo elective refractive surgery for vision correction, although the safety of procedures is relatively high, between 0.04 and 0.6%[11] of patients go on to develop postsurgical ectasias. It is thought that this subset of patients have biomechanically abnormal corneas prior to undergoing surgery, in the form of subclinical keratoconus.

Although corneas are screened extensively prior to refractive surgery using topographic, tomographic, and pachymetric measures, and recently via genetic testing (Avagen™, Avellino Labs USA Inc., CA, USA), none of the current screening tools can reliably identify biomechanical instability. This is confirmed by the presence, in retrospective studies, of patients with corneas that have developed postsurgical ectasia despite being within the normal range in terms of topographic and tomographic measures prior to surgery.[12]

Whilst screening is an important step forward for increasing the safety of refractive surgery, a better understanding of biomechanics is also necessary. Several different procedures are available including: photorefractive keratectomy (PRK), laser-assisted subepithelial keratectomy (LASEK), laser-assisted in-situ keratomileusis (LASIK), and most recently small incision lenticule extraction (SMILE). Since its introduction, LASIK has been the most popular procedure, predominantly due to the epithelium remaining in-tact, facilitating quick recovery, a relative absence of postsurgical pain and low risk of haze, allowing the patient to resume normal activities almost immediately. However, LASIK is the procedure most commonly associated with the development of postsurgical ectasias, accounting for 96% of cases.[11] During LASIK a significantly higher number of collagen fibers in the anterior stroma are severed than in the other procedures. Models have predicted that LASIK results in between 55 and 65% weakening of corneal elastic properties[13] compared to around 20% for PRK.[14]

SMILE is thought to minimize the risks associated with LASIK, whilst delivering equivalent outcomes and the advantages associated with maintenance of the epithelium. In SMILE, a lenticule is cut at a specific depth in the stroma; removal of this

lenticule is achieved via the introduction of two small 2 mm incisions. A reduction in the number of fibers severed in the creation of these small incisions in comparison to the flap in LASIK is thought to offer advantages in terms of biomechanics. In addition, it is thought that removing tissue from deeper in the stroma, preserving the tougher anterior stroma and Bowman's layer, confers further benefits. These presumed biomechanical advantages have been evaluated in studies assessing changes to parameters associated with corneal biomechanical integrity as measured by the Ocular Response Analyser (ORA; Reichert Ophthalmic Instruments, NY, USA) and the Dynamic Schiempflug Tonometer (DST; CorVis ST, OCULUS, Wetzlar, DE). Results have been mixed with some studies showing minimal or no differences[15] but others demonstrating biomechanical benefits of SMILE over LASIK.[16] However, this does not confirm that there are biomechanical advantages in SMILE; it merely highlights the current lack of an adequate means to probe biomechanical parameters, as discussed further in Section 5.

A comprehensive understanding of the spatial biomechanical implications of these procedures would enable the development of methods for minimizing negative biomechanical implications and assist in setting recommended parameters, for example for optimal lenticule depth/thickness, and position/direction of incisions. Interferometric methods have already demonstrated potential for this by quantifying how changes to the angles of incisions can reduce negative strain implications.[17]

Other surgical procedures could also benefit from access to biomechanical information. Nonelective surgeries, including cataract surgery and corneal grafts, are highly invasive and have a profound effect on biomechanics. These surgeries often require stitches in the cornea. The ability to image corneal biomechanics in this instance could both enable stitching to be optimized to avoid uneven tensions that may lead to the development of postoperative astigmatism; and assess the quality of wound healing during the recovery period so that stitches could be removed at the most appropriate time.

Challenges Associated with the Assessment of Biomechanics

Despite an obvious clinical need, driven by the benefits discussed in the previous section, currently no established method exists to reliably assess corneal biomechanics in the clinic, and this is testament to the many challenges associated with their assessment.

Structure and regional variability in biomechanics

The human cornea has a complex collagen structure that varies substantially across its arcs and throughout its thickness.

Through the use of imaging technologies, such as Transmission Electron Microscopy (TEM) and X-ray Diffraction, we have a comprehensive knowledge of the structure of the cornea, described in detail by Meek and Knupp.[18] However, gaps remain in the knowledge of the association of structure with biomechanics.

Some features of corneal structure have been theorized or demonstrated to play a fundamental role in its biomechanical behavior. The stroma, making up over 90% of the thickness of the cornea, is considered in the most part to govern its biomechanics. The anterior stroma is the toughest part of the cornea, consisting of a network of highly interwoven collagen lamellae that insert into Bowman's layer. This mesh-like structure has been demonstrated to provide high tensile strength, being 50% stiffer than the mid or posterior stroma.[19] Due to its structure, the anterior stroma is also resistant to swelling enabling preservation of corneal curvature.[20] In contrast, the collagen across the central cornea in the mid to posterior stroma runs from limbus to limbus and predominantly in two orthogonal directions, along the nasal-temporal and superior-inferior. This arrangement is thought to provide high tensile strength to resist large deformation under the forces imposed by the ocular rectus muscles, which control fine eye movement, and to resist forces imposed by the eyelids during blinking.[21]

As the collagen approaches the peripheral cornea, it transitions to a circumferential arrangement, forming a circumferential annulus at the limbus.[22] In this region, the cornea has been shown to have high circumferential strength while being relatively more compliant in the direction normal to fiber orientation.[23] This specific arrangement has recently been demonstrated to play a key role in how the cornea absorbs small changes in intraocular pressure (IOP) while allowing the curvature of the central cornea to remain relatively unchanged [Fig. 2].[24]

Aside from collagen orientation, collagen crimp has been theorized to play a key role in corneal biomechanics.[18,25–27] Collagen crimp is the term used to describe the natural waviness of collagen fibers under physiological tension; the process of decrimping is thought to contribute significantly to the absorption of IOP fluctuations, through providing an efficient, low energy, and wear resistant deformation mechanism.[28] The biomechanics of collagen crimp in different regions has yet to be explored in detail. Initial studies have identified regional differences in crimp morphology with the limbus showing the largest waviness, tortuosity, and amplitude,[26] and the peripheral cornea showing significantly larger values for the aforementioned features than the central cornea.[27]
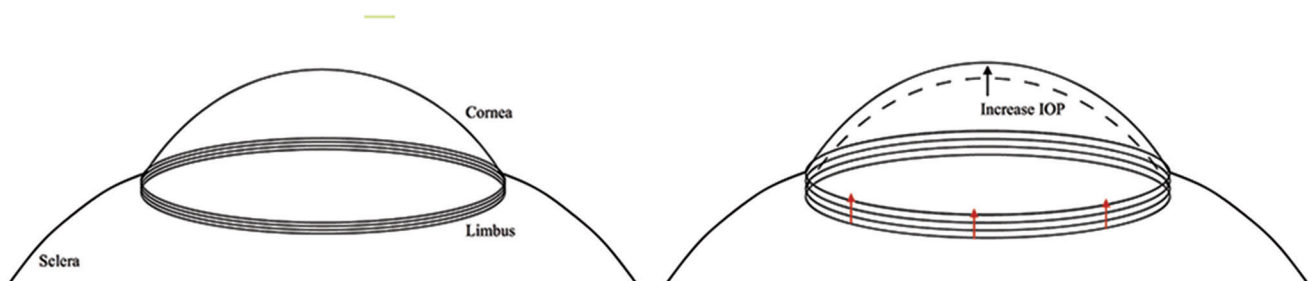

Figure 2: Presumed predominant mode of deformation in response to small pressure perturbations. Reproduced from[24]

A further structural aspect is the presence of elastin fibers. Recent imaging studies have shown that elastin fibers are most concentrated and thickest in the peripheral cornea and posterior limbus[29–31] forming elastic sheets in a layer above Descemet's membrane. Narrow fibers extend from these sheets towards the central cornea. Both the population and elastin content of these fibers have been shown to decrease moving from the limbus to the central cornea.[31] It is postulated that this elastic network acts in tandem with collagen crimp[28] facilitating the absorption of small pressure perturbations as described in Fig. 2.

Nonlinear and viscoelastic behavior

Several ex-vivo studies have confirmed that the cornea exhibits nonlinear behavior.[23,32–34] Demonstrating low stiffness at low pressures and significantly higher stiffness at pressures exceeding those experienced in vivo.[33] This occurs due to the different dominant factors contributing to deformation at each of these states, as summarized in Fig. 3.

In addition to demonstrating nonlinear behavior, the cornea, like many other biological materials, is viscoelastic.[33–36] The response of a viscoelastic material to loading has both an elastic and viscous component; hence, a degree of hysteresis is observed during cyclic loading, as energy is lost, and the cornea takes time to return to its preload state, creep is also observed.[34] The viscoelastic nature of the cornea means that it exhibits different material properties in response to different loading rates, exhibiting higher stiffness in response to increased loading rates.[33]

Variable hydration

The hydration ratio of the cornea (weight of water : dry weight) is approximately 3.2.[37] Changes to hydration have been confirmed to have an effect on biomechanics in several studies.[38–40] This can present issues with regards to the quantitative measurement of mechanical properties both *in vivo* and in laboratory-based experiments. In the laboratory, it is difficult to maintain the tissue at physiological hydration levels, since the majority of corneas will be supplied as corneal-scleral domes that have been stored for some time after removal, leading to gradual loss of function in the endothelial pump and subsequent swelling. Swelling can be reversed via the use of dehydrating agents such as dextran; however, the process of swelling and deswelling may itself lead to permanent changes to the tissue architecture and dextran has been show to introduce other changes that may influence biomechanics.[38] In vivo, the hydration properties

and water content of the cornea do not remain constant throughout the day, varying by on average 7.2% in healthy subjects.[41] Corneal hydration is influenced by factors such as humidity[42], contact lens wear,[43] and age.[44] Hydration levels can also be altered by surgery[45] and in diseased states.[46] This makes evaluating the biomechanical implications of different treatments difficult due to the presence of confounding factors.

Age-related changes and intersubject variability

It is well documented that the stiffness of the cornea increases with age.[47,48] This occurs due to the natural age-related formation of crosslinks in the tissue, and potentially, to some degree, decreases in collagen crimp[26] and changes in elastin content. The stiffening effect is substantial with studies suggesting an approximate doubling in corneal stiffness from 20 to 100 years of age.[48]

As with all biological materials, a degree of intersubject variability is expected, given differences in genetics and ethnicity. Hence, determining the limits that constitute a typical response, or "normal biomechanics," and the factors that may affect it, on top of those already discussed, can be challenging. Especially given the current lack of population data and when trying to identify early stage disease with high sensitivity.

As highlighted by this discussion, quantification of corneal biomechanics is complex and careful consideration must be taken when designing a method to determine corneal biomechanical properties that have relevance to the clinician. The majority of models of corneal biomechanics are currently based on parameters determined from laboratory studies that have used methods that fail to account for the factors described in this section and, as such, do not accurately represent *in vivo* corneal biomechanics. Clinical methods used to probe corneal biomechanics also fail to account for many of these factors which have significant implications when it comes to the diagnosis of abnormality, screening patients for surgical treatments, and facilitating the provision of customized treatments.

Techniques Used for Biomechanical Assessment

Ex-vivo techniques

To-date, almost all quantitative information on the mechanical properties of the cornea has been derived from laboratory-based studies. Most studies undertaken have focussed on determining corneal elasticity, often reporting a single elastic modulus for the cornea. For the reasons discussed in the previous section, this does not provide sufficient information to give a comprehensive understanding of corneal biomechanics, and due to a combination of all the factors discussed, can lead to confusion due to the different nature of elastic moduli reported (Young's, tangent, secant) contributing in-part to the large discrepancies in reported values of corneal elasticity, some of which are summarized in Table 1.

The most common technique used to measure corneal elasticity is strip extensometry, whereby strips of corneal tissue are subjected to tensile forces[49] most commonly uniaxially, but with some recently employing a biaxial approach.[50] While the method has advantages including simplicity, low cost, and the ability to provide a quantitative measure of stiffness, it also has many limitations. Primarily, strips are isolated from the cornea removing the natural state of tension and shape, fundamental
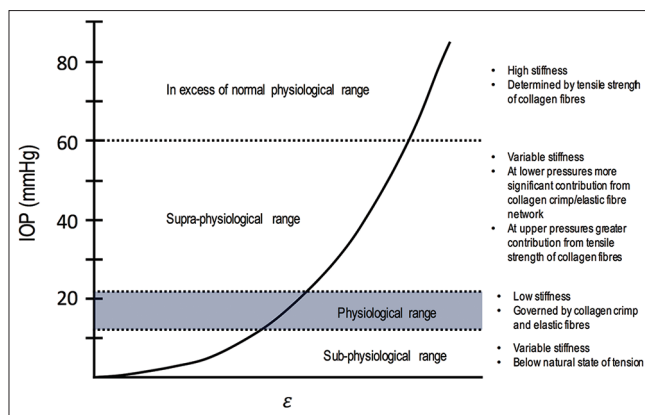

Figure 3: Representative diagram of corneal strain over range of IOP from 0 to 100 mmHg. Different structural components dominate the response at over different ranges of IOP

Table 1: Summary of range of moduli of elasticity quantified using different measurement techniques

Study	Measurement technique	Tissue state	Pressure range (mmHg)	in vivo/ ex vivo	Elastic modulus (MPa)
Hjortdal, (1996)[23]	Particle tracking	Whole eyes	2-100	ex vivo	2.87-27.5 (Regionally and directionally variable); 6.21-13 (physiological range)
Wang, et al. (1996)[92]	Ultrasound (tracking shear wave propagation)	Whole eyes	22	ex vivo	5.3
Wollensak, et al. (2003)[93]	Strip extensometry	Corneal strips	4% strain	ex vivo	0.8
Elsheikh, et al. (2007)[33]	Laser apical displacement tracking	Whole eyes	0-75	ex vivo	0.25-2.75 (pressure dependant); 0.4-1.0 (physiological range)*
Knox-Cartwright, et al. (2011)[48]	Radial shearing interferometry	Intact Corneal-Scleral domes	15.0-15.5	ex vivo	0.27-0.52 (age dependant)
Lombardo, et al. (2014)[94]	Scheimpflug imaging of response to inflation testing	Whole eyes	18-42	ex vivo	0.21 (posterior cornea), 2.28 (anterior cornea)
Shih, et al. (2015)[95]	Scheimpflug imaging of response to air-puff	Whole eyes	~110 during air puff	in vivo	0.01-1.24 (estimated from fitting to custom model)

*Estimated from plotted data

to maintaining corneal biomechanics in vivo. These strips are clamped and loaded in a way that does not account for the natural curvature of the cornea and the nonequal lengths of the anterior and posterior surfaces, introducing a force imbalance; all while the forces applied generally far exceed, in magnitude, those experienced physiologically. Further challenges are found with maintaining a section of corneal tissue at physiological hydration levels; and in clamping the tissue, as variations in clamping position can result in significant variability in response. The effects of these factors are not small, with a study showing a 32% difference in corneal stiffness measured via extensometry versus inflation testing.[49]

Since the limitations of strip extensometry are now widely acknowledged in the ophthalmic research community, many groups have focussed on developing techniques that can be used alongside inflation testing either of whole-eyes or of corneal-scleral domes. Inflation testing more closely simulates physiological forces imposed by IOP fluctuations. Issues are still encountered in terms of maintaining the hydration properties of the tissue after removal from the body, and in recreating the boundary conditions if corneal-scleral domes are used. One of the greatest challenges for laboratory testing is the limited availability of human tissue, meaning studies with human tissue are limited to small sample sizes with many groups opting to use tissue from different species including pigs, cows, sheep, and rabbits. This has driven researchers to focus predominantly on investigating techniques that may have future potential for clinical translation.

Many different methods have been used with inflation testing. In the simplest case, point-based laser displacement tracking has been used to measure the displacement of the corneal apex in response to pressure increases. While this method has effectively demonstrated nonlinearity in corneal response to loading, age-related changes in stiffness, and the effects of viscoelasticity,[33] it provides no information regarding the way in which the cornea deforms and the specific nature of the tissue biomechanics that govern the response. For this, measurement of the deformation of the whole cornea is required. Several techniques have been employed to achieve this including optical coherence tomography (OCT),

digital image correlation (DIC), high-frequency ultrasound imaging (HFU) and interferometric-based techniques. Each of these techniques is summarized in Table 2.

OCT and HFU have the ability to provide 3-D information. OCT lends itself the evaluation of the cornea, due to its high resolution, noninvasive nature. In addition, ophthalmologists are already familiar with its principals due to its use in anterior segment and retinal imaging. As a result, OCT has been trialed in different forms, alongside inflation testing, for the evaluation of corneal elastic properties. Both swept-source OCT and spectral-domain OCT have been used with digital speckle tracking, to measure the 3-D[51] and 2-D[52–54] deformations, respectively, of the cornea in response to pressure changes, as low as 1 mmHg[53] with a displacement resolution of several microns.[51] Using these methods, it has been possible to show through-thickness and regional variability in corneal mechanical properties and even focal regions of abnormality.[3] However, due to several factors including spectral roll-off, transmission of light at the air/specimen interface and material scattering,[51] the ability to reliably track deformation outside the central 6 mm of the cornea is lost, unless the cornea is rotated for measurement. Hence, these methods have not demonstrated efficacy for measuring deformation at the peripheral cornea or the limbus.

Several other limitations exist which have, so far, prevented these methods from being translated to the clinic. The main issue is measurement time because the techniques rely on scanning to build up 2-D and 3-D data; measurement times are of the order of several seconds to several minutes, respectively, which does not present issues in the laboratory, but, in vivo, the eye moves significantly over this time. With advanced eye tracking, it is possible that 2-D clinical data acquisition could be achieved in the near future, with recent methods reported where deformation has been tracked in response to a simulated heartbeat (Hb-OCE).[55] However, if limited to 2-D acquisition, focal abnormalities are likely to be missed. It has also yet to be determined how sensitive the technique is with regards to the detection of subclinical abnormality.

Similar to OCT, HFU has a history of application for anterior segment imaging. HFU provides identical information to OCT

Table 2: Summary of methods used ex vivo and in vivo for the assessment of corneal biomechanics

Measurement technique	in-vivo/ex-vivo	Nature of loading	Measured variables	Spatial resolution	Sensitivity range	Demonstrated ability to detect changes to biomechanics through disease/intervention
Strip extensometry[93]	ex vivo	Tensile testing of corneal strips	Elastic moduli	N/A	N/A	Quantified stiffening effect of CXL[93]
Laser apical displacement tracking[33,47]	ex vivo	Inflation testing	Apical displacement	N/A	1 µm to 1 mm[47]	Sensitivity to age related changes in stiffness[33,47]
OCT[51-54,82-84]	in-vivo/ in vivo potential	Inflation testing (representative of IOP fluctuations induced by heartbeat)[53,55] Tracking of elastic wave[82-84]	3-D displacement Propagation of elastic wave	Through-thickness resolution of Several µm. X-Y resolution dependent on selected scanning frequency	Several nm (Phase-sensitive OCT), to several microns	Quantified changes to through-thickness displacement after crosslinking. (ex vivo).[55] Changes to elastic wave propagation were observed between crosslinked and non-crosslinked regions of cornea (ex vivo).[96] Using displacement tracking in response to pressure changes focal regions of abnormality in a keratoconic cornea were identified (ex vivo).[3]
HFU[56,57,97]	in vivo potential	Inflation testing (representative of IOP fluctuations induced by heartbeat).[56,57] Tracking of shear wave propagation[97]	3-D displacement Propogation of shear wave	Through-thickness resolution of Several µm. X-Y resolution dependent on selected scanning frequency	0.5 µm to 32 µm[57]	Changes to corneal deformation were observed after crosslinking[57]
DIC[34,59]	in vivo potential	Inflation testing	3-D surface displacement	Imaging system dependent, several µm possible	~ 1 µm to several mm	DIC has been used to quantify regional variability in biomechanics[34,59]
Speckle Interferometry[10,17,24,48,63-65]	in vivo potential	Inflation testing (representative of IOP fluctuations induced by heartbeat)	3-D surface displacment	Imaging system dependent, several µm possible	0.01 µm to 3 µm	Interferometry has be used to quantify spatial variability in corneal biomechanics (ex vivo), age-related stiffening of the cornea (ex vivo),[48] changes to corneal stiffness after CXL (ex vivo),[64] spatial changes to the response of corneas to pressure variations after topographic CXL (ex vivo)[10] and changes to the response of the cornea to pressure variations after the introduction of surgical incisions (ex vivo)[17,63]
ORA[66]	in vivo	Air-puff	CH, CRF, CCF	N/A	N/A	CH and CRF are statistically lower in keratoconic corneas,[72] but sensitivity is poor due to significant overlap. Waveform analysis has shown potential for detecting keratoconus at an early stage[73]
DST[67]	in vivo	Air-puff	Cross-sectional spatial and dynamic deformation	~ 10 µm[98]	N/A	Biomechanical index combining several parameters has demonstrated potential for detecting subclinical keratoconus[99]

Table 2: Contd...

Measurement technique	in-vivo/ex-vivo	Nature of loading	Measured variables	Spatial resolution	Sensitivity range	Demonstrated ability to detect changes to biomechanics through disease/intervention
Brillouin spectroscopy[80,100-102]	in vivo	No Loading	3-D Brillouin modulus	X-Y resolution is dependent on number of scanning points (trade-off with acquisition time). Through-thickness resolution of 5 μm.[100]	0.3-16 GHz in Brillouin frequency shift[102]	Brillouin modulus has been demonstrated to change after CXL (ex vivo),[80] and in keratoconus ex vivo[101] and in vivo[79]
Dynamic videokeraoscopy[87]	In vivo	IOP elevations induced by pressure on sclera by means of ophthalmodynamometer[87]	Topography changes	32 placido ring system.	~2.5 to 80 μm	No current evidence

but has reduced spatial resolution and requires direct contact with the cornea via a probe; but due to a larger beam size has an improved tolerance for displacement,[56] which confers benefits when considering clinical translation. Recently, its use has been investigated, in combination with speckle tracking in a technique coined ocular pulse elastography,[56,57] for quantifying the deformation of the cornea in response to pressure perturbations representative in magnitude of those that occur during the cardiac cycle (1.8–4.3 mmHg[58]). HFU was demonstrated to be capable of measuring deformation in response to pressure changes of this scale. However, if clinical translation is to be realized, the effects of eye motion need to be accounted for. Tracking lateral motion is also particularly challenging with HFU due to the nature of the corneal structure,[56] and as with OCT information is only provided for 2-D cross-sections. HFU has in-fact, so far, only demonstrated efficacy for obtaining quantitative data in the very central region (~ 4 mm) of corneal cross-sections.[56,57]

Unlike OCT and HFU, DIC and Interferometry are snapshot methods, where surface information is captured in a single image with no need for scanning. This offers advantages when considering clinical translation, as measurement times are on the scale of milliseconds. The techniques are also less limited in terms of field of view, enabling data capture from the sclera and across the full cornea without the need to move the cornea or for complicated additions to the imaging systems. The main limitation of DIC and interferometric techniques is that they are limited to recording surface information only. Despite preventing full quantification of the mechanical properties, the techniques have been demonstrated to be highly effective for identifying spatial variability in the mechanics of different regions, i.e., the limbus, central and peripheral cornea.[24,34,59] Also, since the cornea can be considered as a thin structure, the surface response represents the bulk deformation of the system, with abnormalities present in superficial layers likely to manifest as abnormalities in surface movement.

As early as 1996, crude versions of DIC were being used to track the movement of particles positioned on different regions of the corneal surface in response to pressure changes.[23,60] Since then, technology has advanced and it is now possible to track the movement of large numbers of particles applied to the corneal surface in 3-D. Boyce, et al. used DIC to quanitify the 3-D movement of the surface of bovine corneas,[34] mounted in an artificial anterior chamber in response to simulated IOP fluctuations, and Whitford, et al., recently used DIC to quantify the deformation of corneas that remained part of the whole eyes in response to IOP changes,[59] with both studies confirming significant spatial variability in response. However, despite technological advancements, due to sensitivity limitations of several micrometers, the use of DIC still requires the cornea to be exposed to pressure fluctuations towards the upper end of the physiological range.

Laser interferometric-based methods manipulate the wave properties of light to measure small surface deformations in high resolution. Early studies used holography to track the deformation of the corneal surface in response to pressure changes of less than 1 mmHg;[61,62] however, these methods were laborios, requiring long image development times. Since then technology has advanced and deformations can be measured digitally, in real time, and quanitified to a resolution of 10's of nanometers. Several different types of

interferometric techniques have been used to probe corneal biomechanics. Jaycock, *et al*., originally used electronic speckle pattern interferometry (ESPI) to measure surface deformation of sheep eyes in response to pressure changes of 0.15 mmHg before and after the introduction of radial incisions,[63] recently followed up by Wilson *et al*., who used ESPI to examine regional variablility in the biomechanical properties of the human corneas[24] and demonstrated the efficacy of the technique for quantifying regional changes to mechanical properties after performing CXL in selected regions.[10] So far this is the first technique that has shown potential for mapping the regional effects of crosslinking; this has significant implications, as with this information treatment models can be tested and validated, potentially facilitating the delivery of targeted CXL to provide accurate refractive outcomes. Clinical translation of the ESPI technique however is problematic, first because the amount of light scattered from the corneal surface is low (around 4%), necessitating the use of mechanisms to enhance reflection to increase signal-to-noise ratio, and second, due to the use of an external reference beam, ESPI is extremely sensitive to vibration and environmental disturbances.

To address the issue of instability, groups have adopted shearographic methods. Speckle shearography works on an identical principle to ESPI; however, instead of using an external reference beam, the signal from the object is split into two halves; one of these halves is transformed in some way, for example, magnified in radial shearing interferometry (RSI); or laterally shifted in lateral shearing interferometry (LSI), and the two parts are interfered on the detector. In this scenario, two points on the object surface, separated by a specific distance, are interfered with each other, removing the influence of external disturbances. The specific setup and nature of interference in shearing interferometry determine the sensitivity. Knox-Cartwright, *et al*., used RSI to examine corneal biomechanics and the effects of aging[48] and CXL;[64] however, full surface quantification of displacement could not be achieved with this method as, due to the nature of interference, sensitivity gradually reduces to zero at the center of the cornea. Wilson, *et al*., recently proposed a LSI approach,[65] using this method whole surface displacement can be determined. An advantage of LSI is that it allows direct imaging of the rate of change of displacement in adjacent regions rather than absolute displacement. This can be advantageous for identifying focal regions of abnormality as it highlights subtle variations in displacement more significantly than when viewing displacement alone. It removes the reliance on specifying boundaries of "normal" displacement, which can be difficult to achieve for biological specimens and instead highlights variations across an individual cornea. However, there are still many challenges to overcome prior to clinical translation, including enhancement of signal-to-noise ratio, increasing stability of the eye over the measurement time, and improving the accuracy of shearographic estimations of displacement.

In-vivo techniques

Currently there are two commercially available instruments for assessment of corneal biomechanics in the clinic; these are the ORA[66] and the DST.[67] Both assess the response of the cornea to an air-puff directed at the center. ORA measures the inward and outward motion of the corneal apex to define corneal hysteresis (CH) – the pressure difference between the inward and outward applanation pressures. DST provides a more comprehensive analysis, through high-speed imaging (4,300 images per second) of the deformation of the central cross-section of a cornea during inward motion and recovery. From this several spatial and dynamic parameters are quantified.

CH is suggested to relate to corneal viscoelasticity, with low-CH values being indicative of corneas less capable of absorbing energy than normal corneas.[68] However, CH has been demonstrated to be influenced by IOP and central corneal thickness so does not independently relate to corneal biomechanics. Attempts have been made to correct for this through the introduction of correction factors when establishing new indicators such as corneal resistance factor (CRF)[69] and corneal constant factor[70] both proposed to relate to corneal rigidity. However, both CH and CRF have been demonstrated to have low sensitivity and specificity for the detection of keratoconus,[71] especially in suspect cases[72] with large overlaps in values between keratoconic and normal corneas. Recently, it has been demonstrated that waveform analysis of the response to the air-puff is a more effective screening tool for keratoconus.[73]

The effectiveness of waveform analysis at a single point is surpassed by the ability to examine a greater number of spatial and dynamic parameters over a cross-section of the cornea with DST. Through concurrent analysis of a number of geometrical and measured parameters Vinciguerra, at al., proposed a new "Biomechanical Index."[74] This approach has successfully demonstrated efficacy for diagnosing keratoconus and even detecting subclinical cases without evidence of topographic or tomographic abnormality.[75]

Use of these tools, alongside recent advancements in artificial intelligence, has further increased the ability of clinicians to identify cases of subclinical keratoconus, as summarized in detail by Lopes *et al*.,[76] and Hogarty *et al*.[77] Employing machine learning algorithms that can combine the wealth of information gained across the whole range of different screening tools improves the chances of identifying abnormalities that may be impossible to recognize when viewing each data set in isolation. Overall saving clinicians significant time and reducing the complexity in decision making, theoretically providing a more comprehensive nonbiased assessment of a patient's suitability for undertaking invasive refractive procedures. However, the effectiveness of all machine learning-based approaches is reliant on; not only the quality of information collected, but the quality of the specific training data sets employed. Recently, machine learning approaches combining topographic and biomechanical data have reported high sensitivities (90.4%) and specificities (96%) for the detection of subclinical keratoconus.[78] However, it has been suggested that in many studies of this nature the distinction between what constitutes subclinical keratoconus and early keratoconus is ambiguous, resulting in false inflations, especially in sensitivity.[77] Most methods employed still have poor sensitivity for truly asymptomatic cases. However, as with most applications of machine learning to biological problems, due to large variability, large training sets are required to gain sufficient accuracy. As this is realized, along with access to improved clinical measurement tools capable of providing information with greater distinguishability characteristics, the effectiveness of these methods will continue to improve.

Despite demonstrating efficacy for detecting the presence of biomechanical abnormality, the current clinical methods used for biomechanics assessments are limited to just that, and do not directly provide information on the mechanical properties of the cornea or the specific position and severity of any area

of focal weakness, hence cannot guide customized treatments. Also, due to the targeted action of the air-puff at the central cornea and the imaging of a central 2-D cross-section only, it would be predicted that such methods of evaluation will have poor sensitivity for identifying corneas with biomechanical weakness in regions away from this. Ultimately, due to the nature of the applied force, the air-puff method does not provide information on the specific biomechanical properties of the cornea important to maintaining its shape and therefore its refractive power. The action of the air-puff is in contradiction to the action of the physiological forces such as IOP, subjecting the anterior surface to bending and the posterior surface to tensile forces. Since the tensile strength of the anterior surface is particularly important to maintaining corneal shape, examining movement of the surface in this way fails to represent its ability to cope with the physiological forces it is adept to dealing with.

Alternative methods for the clinical assessment of elasticity have recently been investigated *in vivo* in small-scale clinical trials. These include Brillouin spectroscopy (BrS), Optical coherence elastography, and dynamic videokeratoscopy. BrS has recently received attention due to its ability to provide 3-D information in the clinic; it has also demonstrated efficacy in laboratory studies for identifying the presence of keratoconus[79] and measuring changes to corneas after undergoing CXL.[80] BrS involves measuring inelastic light scattered from the cornea that arises due to illumination of the tissue initiating thermally excited-hyper-frequency sound waves, which leads to periodic fluctuations in density. The specific frequency shift between the scattered light and the incident light is referred to as the Brillouin modulus, which is related to compressibility in isotropic materials. However, the relationship of Brillouin modulus to mechanical properties becomes complex in hydrated materials, as the measured frequency-shift represents a volume weighted aggregate modulus of the fluid and solid components of the specimen,[81] and therefore there is no direct relationship with elasticity. This is an issue since corneal hydration is variable, both spatially and temporally, and influenced by many different factors. Overall, this has recently led to some researchers cautioning against its use as a tool for optical elastography in biological materials.[81]

Despite this, it should be acknowledged that Brillouin spectroscopy has demonstrated efficacy, not only for detecting the presence of keratoconus but also for providing information regarding the position of abnormality. Its ability to do this may not be due to the fact it identifies changes to the mechanical properties of the tissue but more so that it may identify structural and hydration changes in regions of abnormality. It could therefore prove to be a useful clinical tool if evidence can be provided that it can detect subclinical cases. However, the main barrier to its widespread clinical adoption is long acquisition times, currently it takes several minutes to build a limited-area, low resolution 40-point scan.[79] Hence full-corneal assessment in clinic would currently require several hours.

Several OCT-based approaches have also been trialled in the clinic. Most use variations on phase-sensitive OCT to track the propagation of an elastic wave initiated in the tissue either via a piezoelectric transducer,[82] a focussed micro air-puff[83] or an acoustic wave.[84] Propagation of the wave across and through the tissue is then related to tissue elastic properties via use of elastic-wave models, including: shear wave model, surface wave model, Rayleigh–Lamb frequency model, Leaky Lamb wave, etc., with appropriate quantification of elasticity very much relying on selection of the most fitting model,[85] often with validation carried out on agar phantoms of varying stiffnesses. However, due to the complexities that exist in corneal structure and its properties, none of these models can provide a truly accurate estimation of elasticity, with most reliant upon satisfying assumptions including: uniform thickness and density, homogeneity in structure, and consistent fiber orientation. Aside from this, the short-pulse excitation method used is detached from the normal quasistatic physiological loads to which the cornea is normally exposed; hence, the measured elasticity is unlikely to be representative of tissue elasticity under physiological loading.

Recently, a different variation of OCT was applied in vivo. In this instance, through-thickness corneal deformation was quantified in response to compression of the corneal apex with a flat glass plate.[86] The rationale was that in patients with early stage abnormalities there would be changes in the through-thickness response of the cornea to compression. While the efficacy of this method for identifying early-stage abnormalities in biomechanics remains to be determined, it does not directly quantify biomechanical properties relevant to the maintenance of corneal shape, as similarly to the air-puff it subjects the anterior stroma to a compressive load. Additionally, many patients would be reluctant to have direct pressure applied to their eye via means of a glass plate. However, since spatial evaluation is possible via this method, it may be beneficial for identifying the specific positions of any abnormalities.

Most methods currently employed in the clinic require probing the cornea with nonphysiologically representative forces; hence, it can only be assumed how the measured properties may influence corneal shape or response to normal pressure fluctuations. One group attempted to address this by taking topography measurements with a videokeratoscope before and after increasing IOP.[87] Due to sensitivity limits of the technique, IOP had to be doubled, which was achieved via applying pressure to the sclera using an ophthalmodynamometer. This technique was successful in demonstrating regional variations in properties in response to a doubling of IOP. However, the limited coverage of the videokeratocope required five images to be taken of each eye as the patient looked left, right, up, and down to gain full coverage. The means by which pressure was increased could have resulted in uneven loading. It is also unlikely that, at the current sensitivity limits of several microns, the technique would be capable of picking up subtle changes in biomechanics that may be seen in the early stages of disease, with further work required to determine its capabilities in this regard.

Another approach for determining the biomechanical properties of the cornea, that has been employed in combination with both ex vivo[88] and in vivo[89,90] measurements, is inverse Finite Element Analysis. Finite element models can be particularly useful for modeling the potential effects of procedures such as SMILE, LASIK,[91] or corneal crosslinking. It is easy to change different variables and parameters in the models and compare the outcomes with *in vivo* or ex vivo measurements. This enables different theories about what leads to the observed real-life changes to be conveniently explored. However, all models require assumptions, and since at the base level they are a theoretical extrapolation from empirical data, they can only truly be as comprehensive as the data fed into them. Therefore, improvements to real-world techniques capable of biomechanical assessment are what will really

drive forward improvements to these models. It is likely that in the future, as more advanced techniques are developed for corneal biomechanical assessment, that comprehensive patient-customized models will become available.

Conclusion

In summary, the current lack of a clinical measurement tool capable of spatial analysis of corneal biomechanics with high sensitivity under physiologically relevant forces is a major problem. Diagnosis is lagging behind treatment, as technologies already exist to facilitate customized and optimized treatment of diseases, such as keratoconus, and provide safer and more effective surgical procedures. The potential of these technologies cannot be fully realized until it is possible to accurately and specifically diagnose biomechanical abnormality in clinic; and a more complete understanding of normal corneal biomechanics and the biomechanical effects of surgical interventions is gained.

Laboratory-based technologies have made significant progress, with clear evidence provided that the biomechanics of the cornea are complex, nonlinear, spatially variable, and load dependent. This has led to a clear appreciation for the need to examine corneal biomechanics in response to physiologically relevant forces, driving many groups to focus on techniques capable of measuring the response of the cornea to natural IOP fluctuations. Tracking the deformation of the cornea in response to such forces is particularly useful as it facilitates the understanding of the relationship between biomechanics and the refractive properties of the cornea.

OCT, HFU and Interferometry have all demonstrated potential for measuring the deformation of the cornea in response to physiological-scale IOP fluctuations. The ability of these methods to obtain whole-field information is particularly useful as focal regions of abnormality are easier to identify. This is more effective than determining if elasticity variables lie within a "normal range" because for the many reasons identified in Section 4, the normal range for corneal elasticity may be large with a significant overlap between normal and pathological states.

Many hurdles remain when considering translation of these methods to clinic. Due to eye movement, measurement time is a significant challenge when considering scanning-based techniques such as OCT and HFU, generally limiting them to 2-D through-thickness data acquisition. The snapshot nature of interferometric-based techniques and their already demonstrated ability to highlight regional and local differences in strain with unrivalled sensitivity make them favorable approaches if solutions can be found to increase signal to noise ratio. However, since interferometric techniques measure surface information only, the most complete solution may be a combination of methods – interferometry to show the overall surface response, with OCT or HFU for targeted acquisition of through-thickness information at points of interest. If possible, this could potentially provide a highly effective method to identify and stage the severity of any abnormalities and determine the most appropriate treatment approach.

In the meantime, these methods can and are being utilized effectively in the laboratory; to increase understanding of normal corneal biomechanics and their relationship with structure and shape; to develop treatment algorithms to facilitate the targeted delivery of crosslinking to provide optimized refractive outcomes; and to assess and improve the safety of current refractive surgery procedures.

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